SUPPORTING INFORMATION

Synthetic and Immunological Studies of sTn Derivatives Carrying Substituted Phenylacetylsialic Acids as Cancer Vaccine Candidates

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Synthetic Procedures

General Methods. NMR spectra were recorded on a 400 or 500 MHz instrument with chemical shifts reported in ppm (δ) in reference to Me_4Si if not specified otherwise. Coupling constants (*J*) are reported in hertz (Hz). High resolution electron spray ionization mass spectra (HR ESI MS) were obtained with a Waters Micromass-LCTPremier-XE instrument, and matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) MS were recorded with a Bruker Ultraflex mass spectrometer. Thin layer chromatography (TLC) was performed on silica gel GF254 plates with detection by phosphomolybdic acid in EtOH or by 1% H_2SO_4 in EtOH. Molecular sieves were dried under high vacuum at 170-180 ℃ for 6-10 h immediately before use. Commercial anhydrous solvents and other reagents were used without further purification. The sTn-HSA, sTnNPhAc-KLH, and sTnNPhAc-HSA conjugates were previously prepared.[1]

2-Azidoethyl *O*-[methyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-trifluoroacetamido-D-***glycero*-α-D-***galacto*-2-nonulopyranosylonate]-(2→6)-2-acetamido-2-deoxy-*α*-D-galactopyranoside (5).** A mixture of **3** (1.4 g, 2.37 mmol), **4** (0.71 g, 2.15 mmol), and activated molecular sieves (3 Å, 3.0 g) in anhydrous acetonitrile (10.0 mL) was stirred at rt for 2 h under an atmosphere of argon. After the mixture was cooled to -35 ^{o}C, *N*-iodosuccinamide (NIS) (1.9 g, 8.60 mmol) and triflic acid (TfOH) (76 µL, 0.86 mmol) were added with stirring and the mixture was kept at -35 °C for 0.5 h. The solid material was filtered off and then washed with DCM. The filtrate and washings were combined and extracted with aqueous $Na_2S_2O_3$ and water, dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue was dissolved in 65% HOAc/H_2O (v/v, 20 mL) and heated at 65 °C for 1 h while stirring. The mixture was concentrated and co-evaporated with toluene. The residue was purified by flash column chromatography (CH_2Cl_2/MeOH 25:1) to afford the desired product **5** as a white foamy solid (0.91 g, 52%), R_f 0.12 (CH_2Cl_2/MeOH 16:1) and its *β*-anomer (0.25 g, 14%), R_f 0.15 (CH_2Cl_2/MeOH 16:1). ^{1}H NMR ($CDCl_3$, 400 MHz): *δ* 7.43 (d, *J* 9.6 Hz, 1H, NHCOCF$_3$), 6.06 (d, *J* 8.8 Hz, 1H, NHCOCH$_3$), 5.34 (td, *J* 6.4, 2.4 Hz, 1H, H-8'), 5.30 (dd, *J* 7.6, 2.4 Hz, 1H, H-7'), 4.98 (td, *J* 11.2, 4.8 Hz, 1H, H-4'), 4.86 (d, *J* 4.0 Hz, 1H, H-1), 4.40-4.30 (m, 3H, H-2, H-6' and H-9'a), 4.12-3.99 (m, 2H, H-9'b and H-5'), 3.98-3.92 (m, 2H, 3-OH and OCH$_2$CH$_2$N$_3$), 3.89-3.83 (m, 3H, H-4 and H-6), 3.80 (s, 3H, COOCH$_3$), 3.76-3.69 (m, 1H, H-3), 3.67-3.59 (m, 2H, 4-OH and OCH$_2$CH$_2$N$_3$), 3.53-3.46 (m, 1H, OCH$_2$CH$_2$N$_3$), 3.37-3.30 (m, 2H, H-5 and OCH$_2$CH$_2$N$_3$), 2.61 (dd, *J* 13.2, 4.8 Hz, 1H, H-3'e), 2.11 (s, 2 x 3H, Ac), 2.01, 1.99 (s, 3 x 3H, Ac), 1.93 (t, *J* 12.8 Hz, 1H, H-3'a). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 172.7, 171.2, 171.1, 170.8, 170.1, 168.2, 158.1, 157.7, 117.1, 114.6, 99.0, 98.0, 72.3, 70.6, 69.7, 69.1, 68.7, 68.4, 67.7, 67.5, 63.8, 62.7, 53.3, 50.8, 50.5, 50.0, 37.7, 23.4, 21.2, 20.9, 20.8, 20.7. HR ESI MS (*m*/*z*) calcd. for $C_{30}H_{42}F_3N_5NaO_{18}$ $(M + Na)^+$ 840.2375, found 840.2365.

***N*-{2-*O*-{*O*-[Methyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-trifluoroacetamido-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate]-(2→6)-2-acetamido-2-deoxy-*α*-D-galactopyranosyl}-ethyl} 4-pentenamide (6).** After a solution of **5** (0.4 g, 0.489 mmol) in MeOH (10 mL) was stirred with 10% Pd/C (90 mg) under a H_2 atmosphere at rt overnight, the catalyst was filtered off, and then 4-pentenoic anhydride (0.2 mL, 0.979 mmol) was added at 0 °C to the filtrate. The mixture was stirred at rt overnight. The solution was concentrated under vacuum and the residue was purified by flash chromatography (DCM/MeOH 15:1) to afford **6** as a white foamy solid (0.28 g, 68%). R_f 0.25 (DCM/MeOH 10:1). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.95 (d, *J* 6.4 Hz, 1H, NHCOCH$_3$), 6.58 (d, *J* 9.6 Hz, 1H, NHCOCF$_3$), 6.07 (t, *J* 5.2 Hz, 1H, OCH$_2$CH$_2$NHCO), 5.87-5.76 (m, 1H, CH=CH$_2$), 5.41-5.35 (m, 1H, H-8'), 5.29 (dd, *J* 8.0, 1.6 Hz, 1H, H-7'), 5.10-4.96 (m, 3H, CH=CH$_2$ and H-4'), 4.87 (d, *J* 4.0 Hz, 1H, H-1), 4.77 (br, 1H, 3-OH), 4.34 (dd, *J* 12.0, 2.4 Hz, 1H, H-9'a), 4.29 (dd, *J* 10.8, 2.4 Hz, 1H, H-6'), 4.23-4.16 (m, 1H, H-2), 4.08 (dd, *J* 12.8, 6.0 Hz, 1H, H-9'b), 4.00 (q, *J* 9.6 Hz, 1H, H-5'), 3.91-3.83 (m, 3H, H-4, H-5 and H-6a), 3.82 (s, 3H, COOCH$_3$), 3.77-3.62 (m, 5H, H-3, H-6b and OCH$_2$CH$_2$N$_3$), 3.31-3.23 (m, 1H, OCH$_2$CH$_2$N$_3$), 2.95 (br, 1H, 4-OH), 2.64 (dd, *J* 12.8, 4.8 Hz, 1H, H-3'e), 2.43-2.36 (m, 2H, CH$_2$CH$_2$CH=CH$_2$), 2.34-2.28 (m, 2H, CH$_2$CH$_2$CH=CH$_2$), 2.15, 2.14, 2.13 (s, 3 x 3H, Ac), 2.03, 2.02 (s, 2 x 3H, Ac), 1.95 (t, *J* 12.8 Hz, 1H, H-3'a). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.8, 173.6, 171.3, 171.0, 170.7, 170.0, 168.2, 158.1, 157.8, 137.0, 116.9, 116.0, 114.6, 99.1, 97.8, 72.2, 71.0, 69.5, 69.0, 68.8, 68.2, 67.9, 67.7, 63.8, 62.7, 53.3, 50.8, 49.9, 39.3, 37.7, 35.9, 29.7, 23.0, 21.2, 21.0, 20.9, 20.8. HR ESI MS (*m*/*z*) calcd. for $C_{35}H_{50}F_3N_3NaO_{19}$ $(M + Na)^+$ 896.2888, found 896.2861.

General procedure for the synthesis of compounds 7a-d. To a stirred solution of **6** (180.0 mg, 0.206 mmol) in methanol (4.0 mL) was added a sodium methoxide solution in methanol (0.1 N, 0.4 mL). After the mixture was stirred at rt overnight, it was neutralized with Amberlite 15 (H^+) resin. The resin was filtered off and washed with methanol. The filtrate and the washings were combined and concentrated to a small volume (*ca.* 3.0 mL), and then mixed with an aqueous NaOH solution (1.0 N, 1.0 mL). After the solution was stirred at rt overnight, it was neutralized with Amberlite 15 (H^+) resin. The filtrate and the washings were combined and concentrated under vacuum to give the fully deprotected disaccharide 110 mg [MALDI TOF MS (*m*/*z*) calcd. for $C_{24}H_{40}N_3Na_2O_{14}$ $(M - H + 2Na)^+$, 640.23, found 640.10], which was used directly in the next step without further purification. After the resultant disaccharide (28.0 mg, 0.047 mmol) was dissolved in MeOH (4.0 mL), a NaOH aqueous solution (1 N) was added to adjust the pH value of the solution to slightly basic, and then an individual acyl anhydride, i.e. (*p*-methylphenylacetic, *p*-methoxyphenylacetic, *p*-acetylphenylacetic or *p*-chlorophenylacetic anhydrides, 0.19 mmol), was added at 0 °C. After the reaction finished as shown by TLC, the mixture was concentrated under vacuum. The residue was dissolved in a small amount of H_2O, extracted with AcOEt to

remove any less polar materials and the product was then purified on a Biogel P-2 column using distilled water as the eluent to give **7a-d** as a white solid (30-33 mg) after lyophilization.

N-{**2-*O*-{*O*-[3,5-Dideoxy-5-(*p*-methylphenylacetamido)**-D-***glycero***-α-D-***galacto***-2-nonulopyranosylonic acid]-(2→6)-2-acetamido-2-deoxy-*α*-D-galactopyranosyl}-ethyl} 4-pentenamide (7a).** ^{1}H NMR (D_2O, 400 MHz): *δ* 7.22 (s, 4H, aromatic H), 5.89-5.77 (m, 1H, $\underline{CH}$=CH_2), 5.11-4.99 (m, 2H, CH=$\underline{CH_2}$), 4.13 (dd, *J* 11.6, 3.6 Hz, 1H, H-2), 3.95 (br, 2H), 3.92-3.64 (m, 8H), 3.64-3.44 (m, 6H), 3.33-3.24 (m, 2H), 2.70 (dd, *J* 12.0, 4.0 Hz, 1H, H-3'e), 2.38-2.32 (m, 4H, CO$\underline{CH_2CH_2}$CH=CH_2), 2.31 (s, 3H, Ph$\underline{CH_3}$), 2.02 (s, 3H, NHCO$\underline{CH_3}$), 1.66 (t, *J* 12.4 Hz, 1H, H-3'a). ^{13}C NMR (D_2O, 100 MHz): δ 176.6, 176.1, 174.8, 173.4, 137.8, 137.3, 132.2, 129.8, 129.3, 115.9, 100.4, 97.4, 72.8, 72.0, 69.8, 68.7, 68.6, 68.1, 68.0, 66.9, 63.9, 63.1, 52.1, 50.0, 42.5, 40.5, 39.2, 35.3, 29.7, 22.3, 20.4. HR ESI MS (*m*/*z*) calcd. for $C_{33}H_{48}N_3Na_2O_{15}$ $(M - H + 2Na)^+$ 772.2881, found 772.2878.

N-{**2-*O*-{*O*-[3,5-Dideoxy-5-(*p*-methoxyphenylacetamido)**-D-***glycero***-α-D-***galacto***-2-nonulopyranosylonic acid]-(2→6)-2-acetamido-2-deoxy-*α*-D-galactopyranosyl}-ethyl} 4-pentenamide (7b).** ^{1}H NMR (D_2O, 400 MHz): *δ* 7.26 (d, *J* 8.8 Hz, 2H, aromatic H), 6.98 (d, *J* 8.8 Hz, 2H, aromatic H), 5.89-5.78 (m, 1H, $\underline{CH}$=CH_2), 5.11-4.99 (m, 2H, CH=$\underline{CH_2}$), 4.82 (d, *J* 3.2 Hz, 1H, H-1), 4.13 (dd, *J* 11.6, 4.0 Hz, 1H, H-2), 3.98-3.94 (m, 2H), 3.91-3.84 (m, 1H), 3.82 (s, 3H, PhO$\underline{CH_3}$), 3.83-3.77 (m, 3H), 3.76-3.58 (m, 5H), 3.57-3.45 (m, 5H), 3.33-3.25 (m, 2H), 2.71 (dd, *J* 12.0, 4.4 Hz, 1H, H-3'e), 2.38-2.30 (m, 4H, CO$\underline{CH_2CH_2}$CH=CH_2), 2.02 (s, 3H, NHCO$\underline{CH_3}$), 1.65 (t, *J* 12.4 Hz, 1H, H-3'a). ^{13}C NMR (D_2O, 100 MHz): δ 176.6, 176.2, 174.8, 173.6, 158.3, 137.2, 130.6, 127.9, 115.9, 114.7, 100.6, 97.4, 72.8, 72.1, 69.8, 68.7, 68.6, 68.2, 68.0, 66.8, 63.9, 63.0, 55.7, 52.2, 50.0, 42.0, 40.7, 39.2, 35.3, 29.7, 22.3. HR ESI MS (*m*/*z*) calcd. for $C_{33}H_{48}N_3Na_2O_{16}$ $(M - H + 2Na)^+$ 788.2830, found 788.2827.

N-{**2-*O*-{*O*-[5-(*p*-Acetylphenylacetamido)-3,5-dideoxy**-D-***glycero***-α-D-***galacto***-2-nonulopyranosylonic acid]-(2→6)-2-acetamido-2-deoxy-*α*-D-galactopyranosyl}-ethyl} 4-pentenamide (7c).** ^{1}H NMR (D_2O, 400 MHz): *δ* 7.96 (d, *J* 8.8 Hz, 2H, aromatic H), 7.45 (d, *J* 8.0 Hz, 2H, aromatic H), 5.89-5.76 (m, 1H, $\underline{CH}$=CH_2), 5.10-4.99 (m, 2H, CH=$\underline{CH_2}$), 4.82 (d, *J* 3.2 Hz, 1H, H-1), 4.14 (dd, *J* 12.0, 3.6 Hz, 1H, H-2), 3.98-3.93 (m, 2H), 3.92-3.64 (m, 10H), 3.63-3.45 (m, 4H), 3.34-3.25 (m, 2H), 2.72 (dd, *J* 12.8, 4.8 Hz, 1H, H-3'e), 2.65 (s, 3H, $\underline{CH_3}$COPh), 2.39-2.32 (m, 4H, CO$\underline{CH_2CH_2}$CH=CH_2), 2.02 (s, 3H, NHCO$\underline{CH_3}$), 1.65 (t, *J* 12.4 Hz, 1H, H-3'a). ^{13}C NMR (D_2O, 100 MHz): δ 203.9, 176.6, 174.9, 174.7, 173.7, 141.6, 138.6, 137.2, 135.7, 129.7, 129.4, 128.9, 115.9, 100.6, 97.4, 72.8, 72.1, 69.8, 68.7, 68.6, 68.3, 68.0, 66.8, 63.9, 62.9, 52.2, 50.0, 42.8, 40.7, 39.2, 35.3, 29.7, 26.5, 22.3. HR ESI MS (*m*/*z*) calcd. for $C_{34}H_{48}N_3Na_2O_{16}$ $(M - H + 2Na)^+$ 800.2830, found 800.2807.

***N*-{2-*O*-{*O*-[5-(*p*-Chlorophenylacetamido)-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid]-(2→6)-2-acetamido-2-deoxy-α-D-galactopyranosyl}-ethyl} 4-pentenamide (7d).** ^{1}H NMR (D_2O, 400 MHz): δ 7.37 (d, *J* 8.0 Hz, 2H, aromatic H), 7.27 (d, *J* 8.0 Hz, 2H, aromatic H), 5.88-5.77 (m, 1H, $\underline{CH}$=CH_2), 5.10-4.98 (m, 2H, CH=$\underline{CH_2}$), 4.82 (d, *J* 4.0 Hz, 1H, H-1), 4.13 (dd, *J* 11.2, 4.0 Hz, 1H, H-2), 3.98-3.93 (m, 2H), 3.92-3.74 (m, 5H), 3.74-3.63 (m, 3H), 3.62-3.43 (m, 6H), 3.32-3.24 (m, 2H), 2.71 (dd, *J* 12.8, 4.8 Hz, 1H, H-3'e), 2.38-2.29 (m, 4H, CO$\underline{CH_2}\underline{CH_2}$CH=$CH_2$), 2.01 (s, 3H, NHCO$\underline{CH_3}$), 1.64 (t, *J* 12.0 Hz, 1H, H-3'a). ^{13}C NMR (D_2O, 100 MHz): δ 176.5, 175.5, 174.7, 173.6, 137.2, 133.9, 132.8, 130.9, 129.1, 128.8, 115.9, 100.6, 97.4, 72.8, 72.1, 69.8, 68.7, 68.6, 68.3, 68.0, 66.8, 63.9, 63.0, 52.2, 50.0, 42.2, 40.7, 39.2, 35.3, 29.7, 22.3. HR ESI MS (*m*/*z*) calcd. for $C_{32}H_{45}ClN_3Na_2O_{15}$ $(M - H + 2Na)^+$ 792.2335, found 792.2335.

General procedure for the synthesis of compounds 8a-d. To the stirred solution of **7a-d** (18 mg) in MeOH (5 mL) at -78 °C, ozone was bubbled until a blue color appeared and remained at -78 °C for 0.5 h. After introducing nitrogen to remove the remaining ozone, Me_2S (0.5 mL) was added at -78 °C. The resultant solutions were allowed to warm to rt over a period of 1 h and stand for another 1 h before it was condensed in vacuum. The crude products were purified by a Biogel P-2 column using distilled water as the eluent to give the aldehydes after lyophilization as white solids, which were used in the following conjugation reactions without further purification.

***N*-{2-*O*-{*O*-[3,5-Dideoxy-5-(*p*-methylphenylacetamido)-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid]-(2→6)-2-acetamido-2-deoxy-α-D-galactopyranosyl}-ethyl} 4-oxo-butanamide (8a).** ^{1}H NMR (D_2O, 500 MHz): δ 7.12 (s, 4H, aromatic H), 5.34-5.23 (m, 1H, -$\underline{CH}(OH)_2$), 4.74 (d, *J* 3.5 Hz, 1H, H-1), 4.05-3.99 (m, 1H), 3.29-3.14 (m, 2H), 2.21 (s, 3H, Ph$\underline{CH_3}$), 1.91 (s, 3H, NHCO$\underline{CH_3}$). HR ESI MS (*m*/*z*) calcd. for $C_{32}H_{46}N_3O_{16}$ $(M - H)^+$ 728.2878, found 728.2861.

***N*-{2-*O*-{*O*-[3,5-Dideoxy-5-(*p*-methoxyphenylacetamido)-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid]-(2→6)-2-acetamido-2-deoxy-α-D-galactopyranosyl}-ethyl} 4-oxo-butanamide (8b).** ^{1}H NMR (D_2O, 500 MHz): δ 7.18 (d, *J* 8.0 Hz, 2H, aromatic H), 6.89 (d, *J* 8.0 Hz, 2H, aromatic H), 5.37-5.23 (m, 1H, -$\underline{CH}(OH)_2$), 4.74 (d, *J* 3.5 Hz, 1H, H-1), 4.07-3.98 (m, 1H), 3.70 (s, 3H, PhO$\underline{CH_3}$), 1.91 (s, 3H, NHCO$\underline{CH_3}$). HR ESI MS (*m*/*z*) calcd. For $C_{32}H_{46}N_3O_{17}$ $(M - H)^+$ 744.2827, found 744.2822.

***N*-{2-*O*-{*O*-[5-(*p*-Acetylphenylacetamido)-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid]-(2→6)-2-acetamido-2-deoxy-α-D-galactopyranosyl}-ethyl} 4-oxo-butanamide (8c).** ^{1}H NMR (D_2O, 500 MHz): δ 7.85 (d, *J* 8.5 Hz, 2H, aromatic H), 7.34 (d,

J 8.5 Hz, 2H, aromatic H), 5.34-5.23 (m, 1H, -CH(OH)$_2$), 4.73 (d, *J* 3.5 Hz, 1H, H-1), 4.05-3.98 (m, 1H), 3.90-3.54 (m, 12H), 3.54-3.43 (m, 2H), 3.42-3.33 (m, 2H), 3.26-3.18 (m, 2H), 2.53 (s, 3H, PhCOCH$_3$), 1.91 (s, 3H, NHCOCH$_3$). HR ESI MS (*m*/*z*) calcd. for $C_{33}H_{46}N_3Na_2O_{17}$ (M - H + 2Na)$^+$ 802.2623, found 802.2612.

***N*-{2-*O*-{*O*-[5-(*p*-Chlorophenylacetamido)-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid]-(2→6)-2-acetamido-2-deoxy-*α*-D-galactopyranosyl}-ethyl} 4-oxo-butanamide (8d).** ^{1}H NMR (D_2O, 500 MHz): *δ* 7.28 (d, *J* 8.5 Hz, 2H, aromatic H), 7.17 (d, *J* 8.5 Hz, 2H, aromatic H), 5.34-5.23 (m, 1H, -CH(OH)$_2$), 4.73 (d, *J* 3.5 Hz, 1H, H-1), 4.06-3.99 (m, 1H), 3.90-3.35 (m, 16H), 3.27-3.16 (m, 2H), 1.91 (s, 3H, NHCOCH$_3$). HR ESI MS (*m*/*z*) calcd. for $C_{31}H_{43}ClN_3Na_2O_{16}$ (M - H + 2Na)$^+$ 794.2127, found 794.2136.

General procedure for the coupling 8a-d to KLH and HSA. A solution of **8a-d** (7 mg), KLH or HSA (7 mg), and $NaBH_3CN$ (7 mg) in 0.1 M $NaHCO_3$ solution (0.1 mL, pH 7.5-8.0) was allowed to stand at rt in the dark for 4 days with occasional shaking. The reaction mixture was then purified with a Biogel A 0.5 column using 0.1 M PBS buffer (*I* = 0.1, pH =7.8) as the eluent. Fractions containing the glycoconjugate, as characterized by the bicinchoninic acid (BCA) assay for proteins and the resorcinol assay for sialic acid, were combined and dialyzed against distilled water for 2 days. The solution was then lyophilized to afford white solids of the desirable glycoconjugates **1a-d** and **2a-d** (*ca.* 6-7 mg).

Analysis of the carbohydrate loading levels of glycoconjugates 1a-d and **2a-d**.[2] The solution of an exactly weighed glycoconjugate (0.35-0.6 mg) in distilled water (1.0 mL) was mixed with the resorcinol reagent (2.0 mL), and the mixture was heated in a boiling water bath for 30 min. It was cooled to rt, and to the solution was then added an extraction solution (1-butanol acetate and 1-butanol, 85:15 v/v, 3.0 mL). The mixture was shaken vigorously before it was allowed to stand still for *ca.* 10 min to allow the organic and inorganic layers to be separated well. The organic layer was transferred to a 1.0-cm cuvette, and its absorbance at 580 nm was determined with an UV-Vis spectrometer, utilizing a blank extraction solution as the control. The sialic acid contents of the glycoconjugates were determined against a calibration curve created with the solution of individual standard NeuNPhAc derivatives analyzed under the same condition. The carbohydrate loading of each glycoconjugate was calculated according to the following equation, and the results are shown in Table 1.

$$\text{Carbohydrate loading (\%)} = \frac{\text{carbohydrate content (mg) in the sample}}{\text{weight of the glycoconjugate sample (mg)}} \text{ x } 100\%$$

Table 1. Antigen Loading Levels of the Conjugates

sample	KLH conjugates				HSA conjugates			
	1a	**1b**	**1c**	**1d**	**2a**	**2b**	**2c**	**2d**
Loading (%)	3.6	4.3	2.9	5.4	5.8	7.6	5.7	6.9

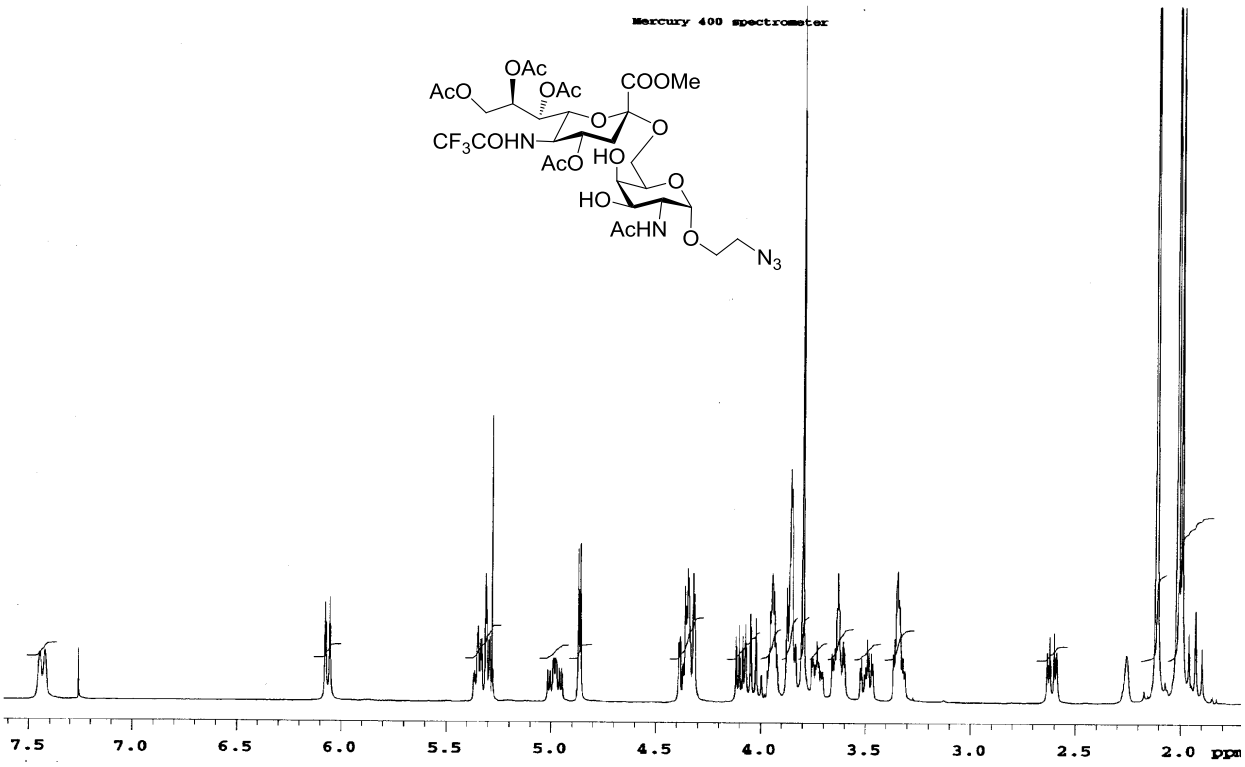
References

1. Wang, Q.; Ekanayaka, S. A.; Wu, J.; Zhang, J.; Guo, Z. Synthetic and Immunological Studies of 5'-N-Phenylacetyl sTn to Develop Carbohydrate-Based Cancer Vaccines and to Explore the Impacts of Linkage between Carbohydrate Antigens and Carrier Proteins. *Bioconjugate Chemistry* **2008,** 19, 2060-2067.
2. Svennerholm, L. Quantitative estimation of sialic acids. II. A colorimetric resorcinol-hydrochloric acid method. *Biochim Biophys Acta* **1957,** 24, 604-11.

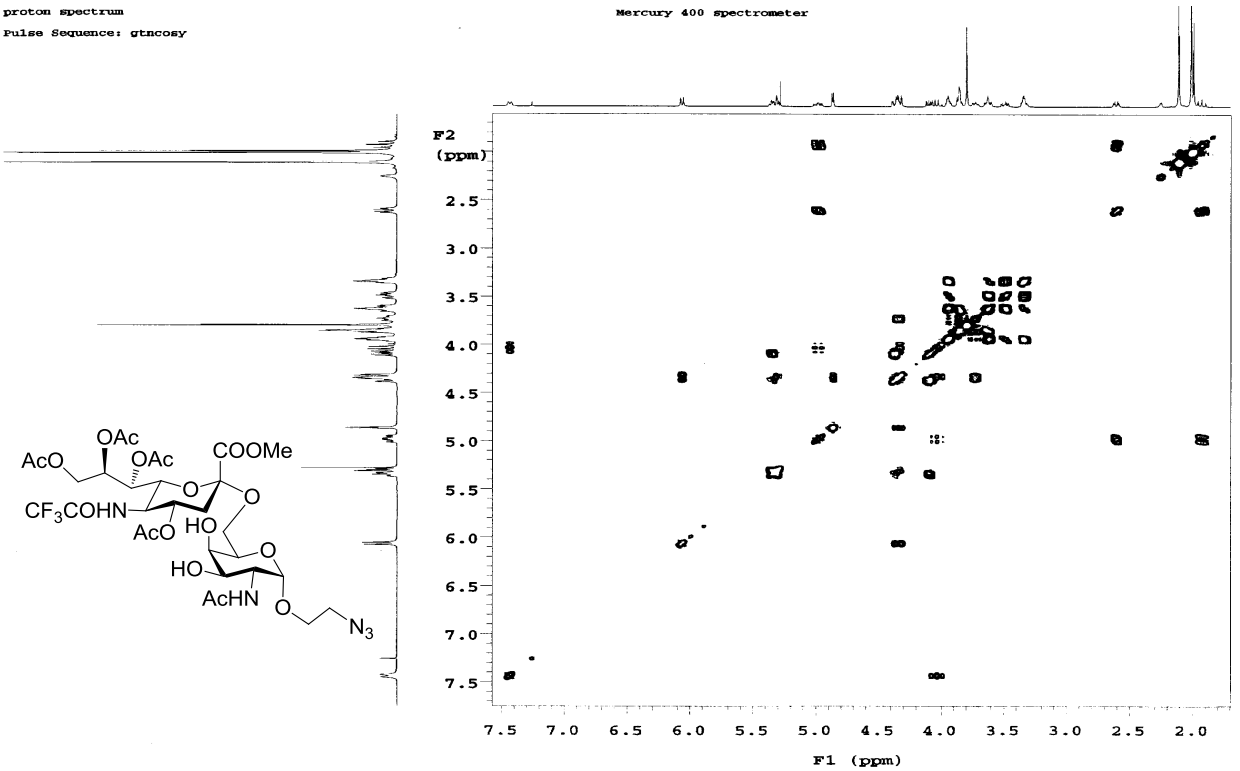
Immunological Studies

Immunization of Mice. Groups of female C57BL/6 mice (5 per group) at the age of 6-8 weeks (Jackson Laboratories, Bar Harbor, ME) were immunized into 3 intramuscular sites with a total of 0.1 mL of the emulsion of **1a-d** (containing 2 μg of carbohydrate antigen) and Titermax Gold adjuvant (Sigma Chemical, St. Louis, MO) on day 0, 14, 21 and 28, respectively. The mice were bled on day -1 prior to the initial immunization and after immunization on day 27 and day 37. Blood samples collected at each time point were clotted to obtain antisera and stored at -80 ^{o}C.

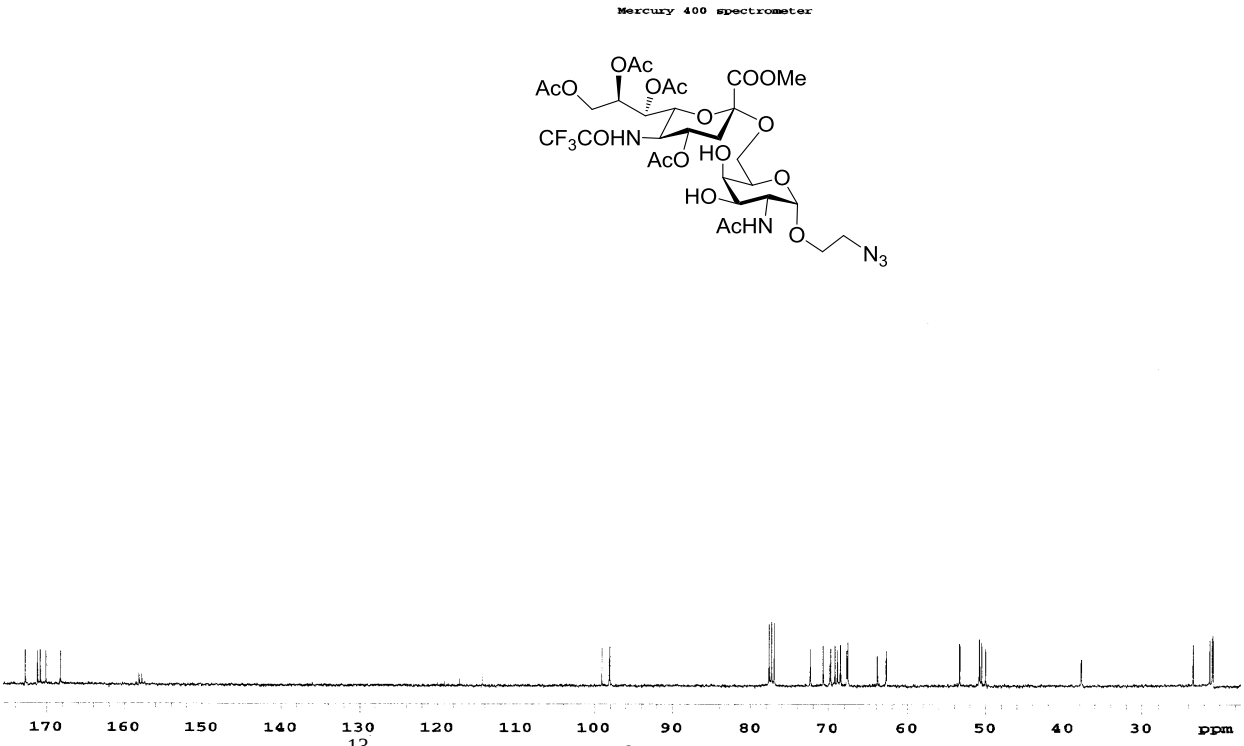
Enzyme-Linked Immunosorbent Assay (ELISA). ELISA plates were first treated respectively with 100 μL of sTn-HSA, sTnNPhAc-HSA or conjugate **2a-d** solution (2 μg/mL) in the coating buffer (0.1 M bicarbonate, pH 9.6) overnight at 4 °C, followed by washing 3 times with PBS containing 0.05% Tween-20 (PBST). Individual or pooled antisera from **1a-d** inoculated mice were diluted 1:300 to 1:72900 in serial half-log dilutions in PBS and incubated for 2 h at 37 ^{o}C in the coated ELISA plates (100 μL/well). The plates were then washed and incubated with 1:1000 dilution of alkaline phosphatase linked goat anti-mouse kappa, IgM or IgG2a antibody or with a 1:2000 dilution of alkaline phosphatase linked goat anti-mouse IgG1 or IgG3 antibody for 1 h at rt. Finally, the plates were washed and developed with 100 μL of PNPP solution (1.67 mg/mL in PNPP buffer) for 30 min at rt for colorimetric readout using a BioRad 550 plate reader at 405 nm wavelength. For titer analysis, optical density (OD) values were plotted against dilution values, and a best-fit line was obtained. The equation of this line was employed to calculate the dilution value at which an OD of 0.5 was achieved, and the antibody titer was calculated at the inverse of this dilution value.



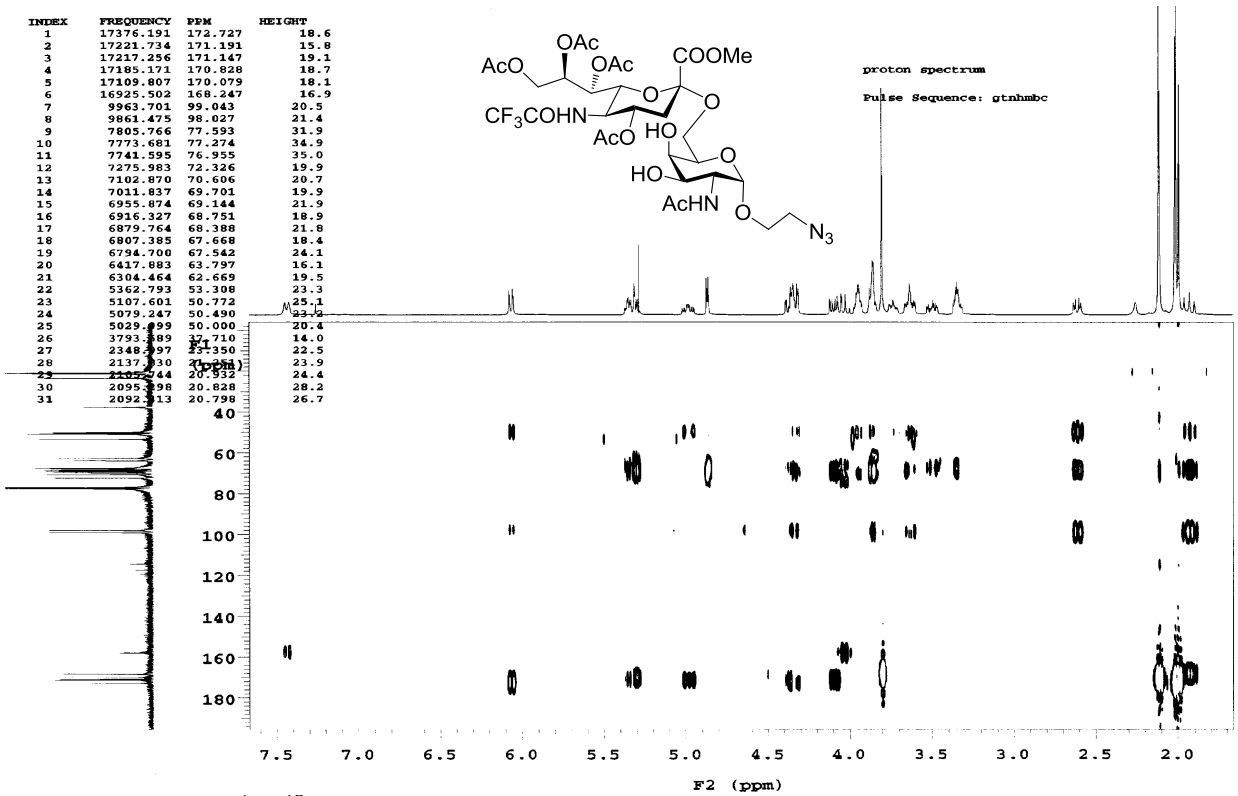
^{1}H NMR spectrum of **5** ($CDCl_3$, 400 MHz)



^{1}H-^{1}H COSY NMR spectrum of **5** ($CDCl_3$, 400 MHz)

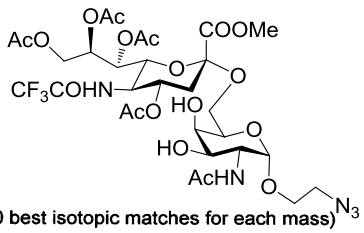


^{13}C NMR spectrum of **5** ($CDCl_3$, 100 MHz)



^{1}H-^{13}C HMBC NMR spectrum of **5** ($CDCl_3$, 400/100 MHz)

Elemental Composition Report

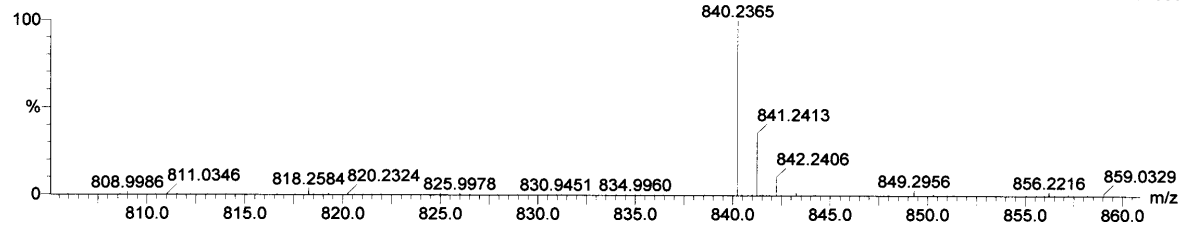


Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
3063 formula(e) evaluated with 24 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-500 H: 0-1000 N: 3-6 O: 10-20 23Na: 0-1 F: 0-4

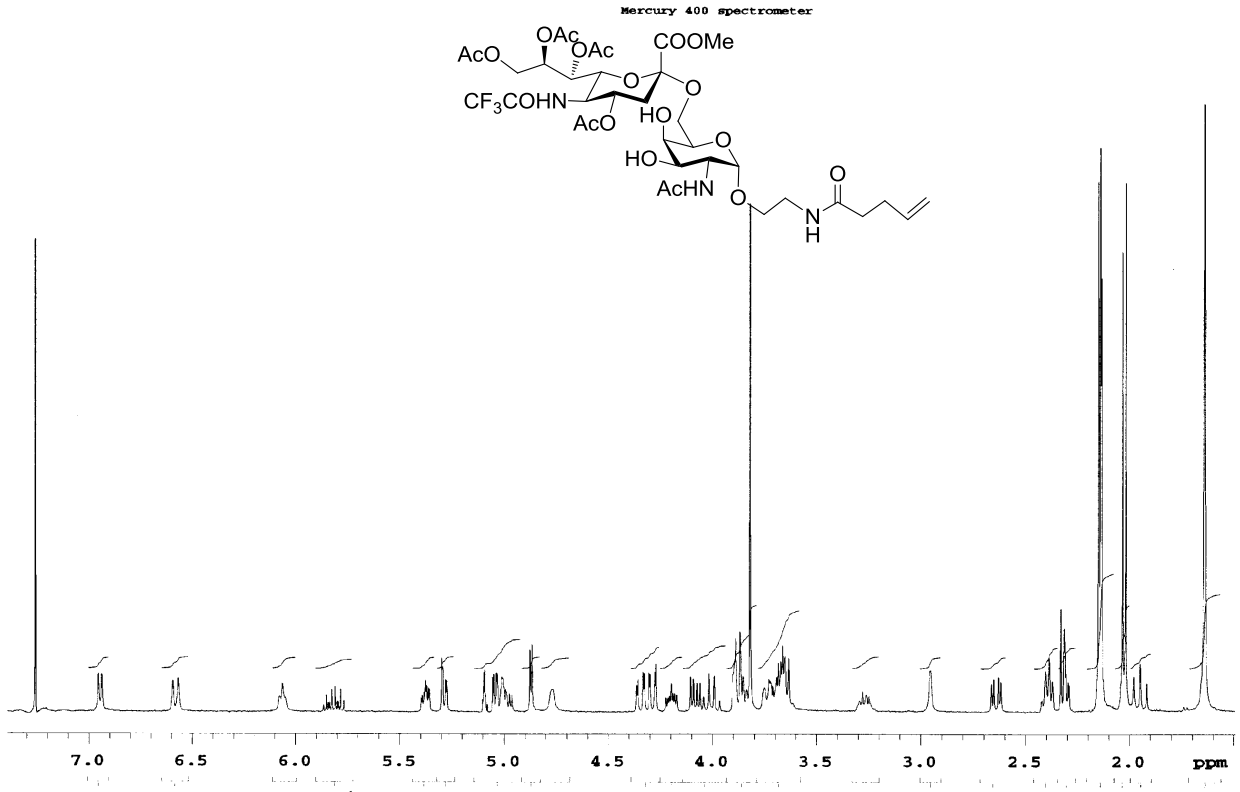
Q. WANG
2008-07b.pro
2009_0710_0487 13 (0.283) Cm (12:16-(1:7+28:34)x4.000)

LCT Premier 10-Jul-2009 14:48:52
1: TOF MS ES+
3.50e+003

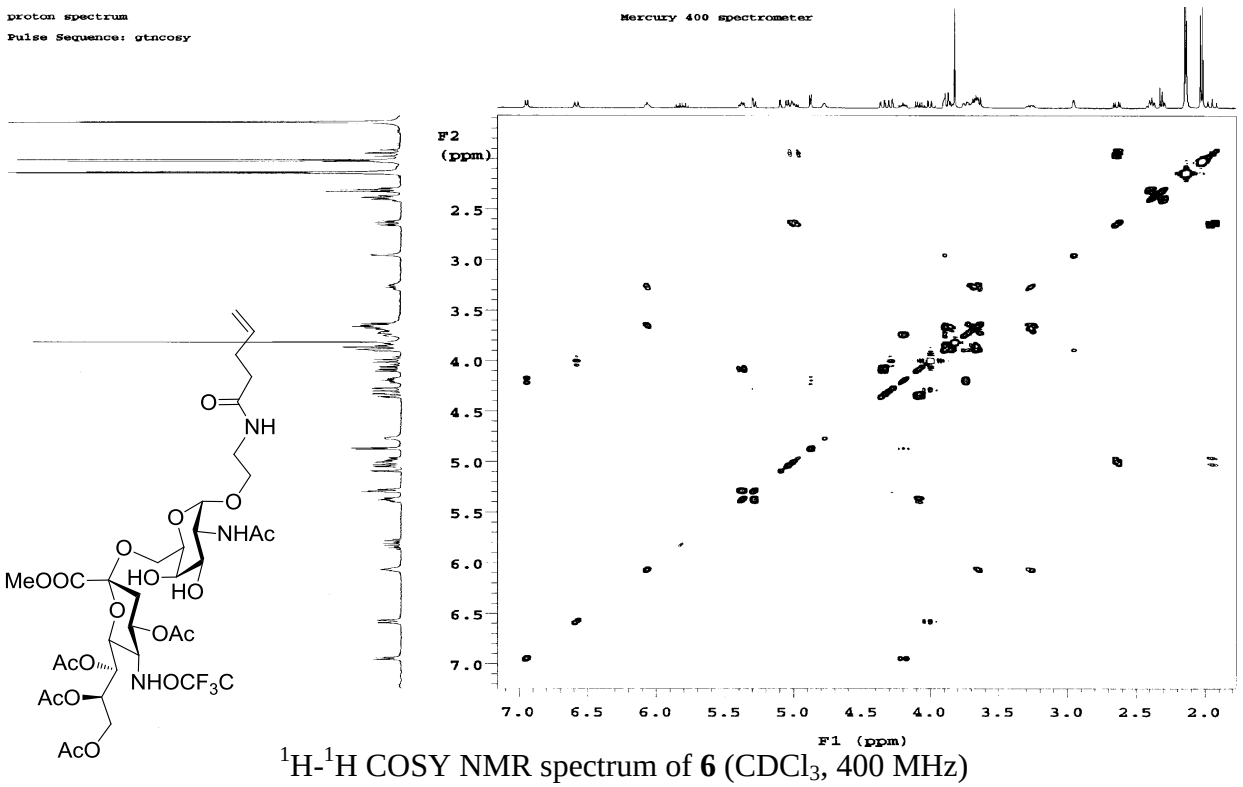


Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
840.2365	840.2375	-1.0	-1.2	10.5	54.5	0.3	C30 H42 N5 O18 23Na F3
	840.2386	-2.1	-2.5	6.5	56.5	2.2	C27 H43 N5 O19 23Na F4
	840.2363	0.2	0.2	14.5	57.3	3.1	C33 H41 N5 O17 23Na F2
	840.2399	-3.4	-4.0	13.5	57.7	3.4	C32 H41 N5 O18 F3
	840.2399	-3.4	-4.0	13.5	58.1	3.9	C32 H43 N5 O20 23Na
	840.2387	-2.2	-2.6	17.5	59.6	5.4	C35 H40 N5 O17 F2
	840.2351	1.4	1.7	18.5	59.9	5.7	C36 H38 N5 O14 F4
	840.2352	1.3	1.5	18.5	60.0	5.8	C36 H40 N5 O16 23Na F
	840.2327	3.8	4.5	15.5	60.2	6.0	C34 H39 N5 O14 23Na F4
	840.2368	-0.3	-0.4	19.5	60.6	6.3	C39 H39 N3 O12 23Na F4
	840.2376	-1.1	-1.3	21.5	60.8	6.5	C38 H39 N5 O16 F
	840.2364	0.1	0.1	25.5	61.8	7.5	C41 H38 N5 O15
	840.2356	0.9	1.1	23.5	61.9	7.7	C42 H38 N3 O11 23Na F3
	840.2403	-3.8	-4.5	18.5	62.1	7.9	C38 H41 N3 O15 23Na F2
	840.2340	2.5	3.0	22.5	62.2	8.0	C39 H37 N5 O13 F3
	840.2340	2.5	3.0	22.5	62.3	8.0	C39 H39 N5 O15 23Na
	840.2392	-2.7	-3.2	22.5	62.4	8.1	C41 H38 N3 O12 F4
	840.2392	-2.7	-3.2	22.5	62.5	8.3	C41 H40 N3 O14 23Na F
	840.2380	-1.5	-1.8	26.5	62.7	8.4	C44 H37 N3 O11 F3
	840.2381	-1.6	-1.9	26.5	62.7	8.5	C44 H39 N3 O13 23Na
	840.2369	-0.4	-0.5	30.5	63.1	8.9	C47 H36 N3 O10 F2
	840.2345	2.0	2.4	27.5	63.5	9.2	C45 H37 N3 O10 23Na F2
	840.2329	3.6	4.3	26.5	64.1	9.8	C42 H36 N5 O12 F2
	840.2405	-4.0	-4.8	29.5	64.9	10.7	C46 H38 N3 O13

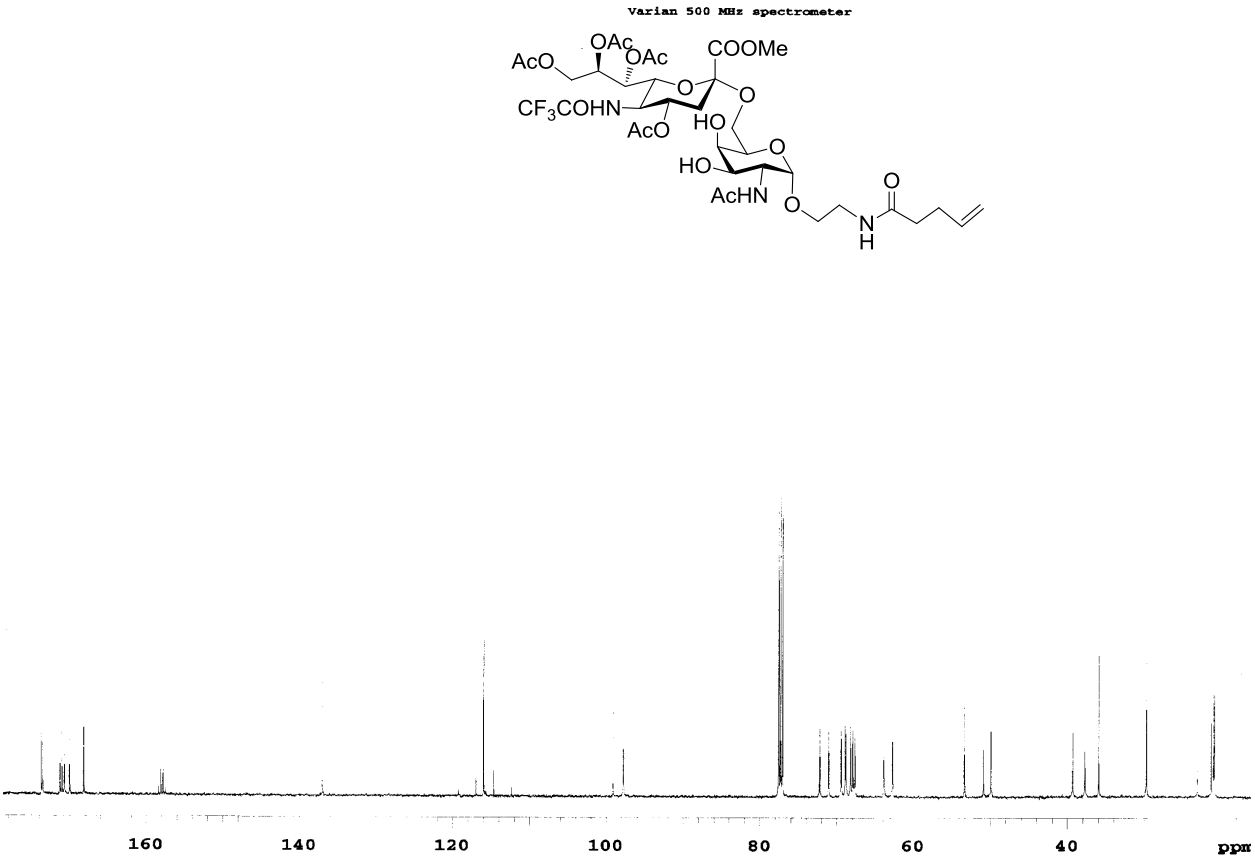
HR ESI MS spectrum of **5**



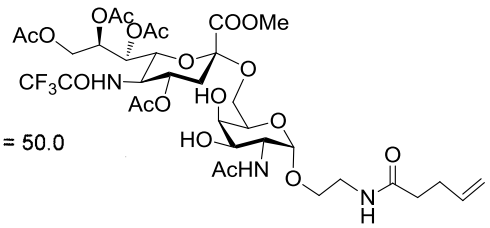
^{1}H NMR spectrum of **6** ($CDCl_3$, 400 MHz)



^{1}H-^{1}H COSY NMR spectrum of **6** ($CDCl_3$, 400 MHz)



^{13}C NMR spectrum of **6** ($CDCl_3$, 100 MHz)



Elemental Composition Report

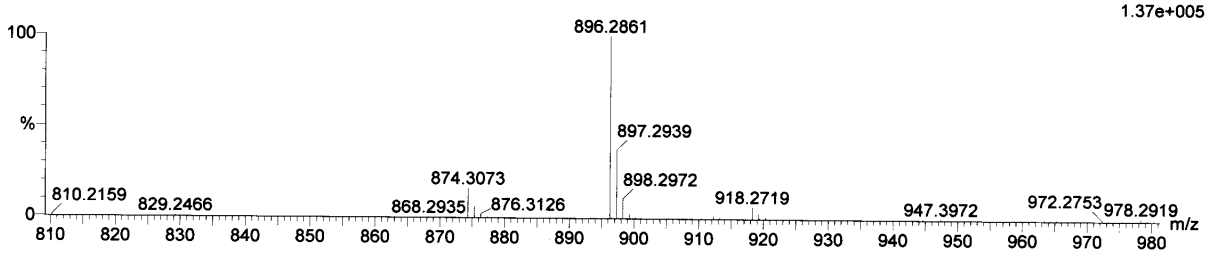
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
6232 formula(e) evaluated with 39 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-50 H: 0-100 N: 0-5 O: 0-21 23Na: 0-1 F: 0-4

2009_0716_0497 13 (0.283) Cm (10:18-(1:7+40:45)x2.000)
LCT Premier 16-Jul-2009 13:33:52
1: TOF MS ES+

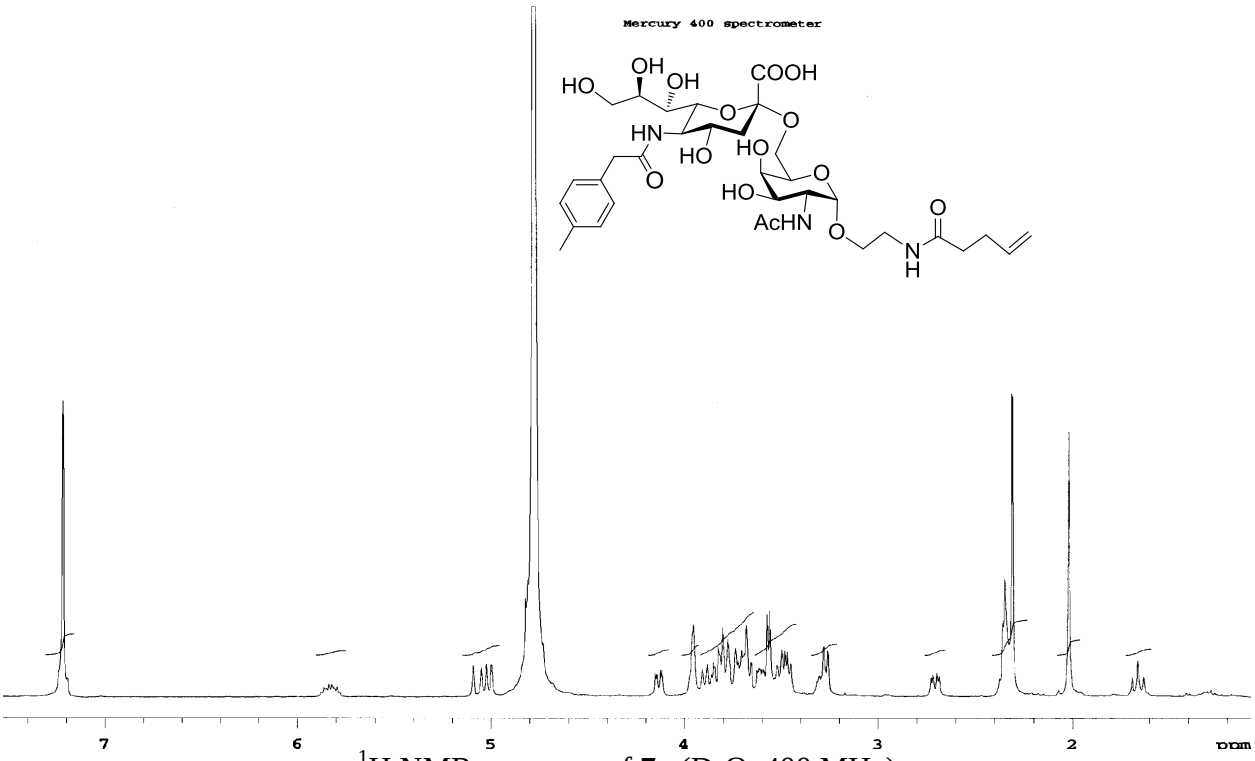
Q. WANG [redacted]

1.37e+005

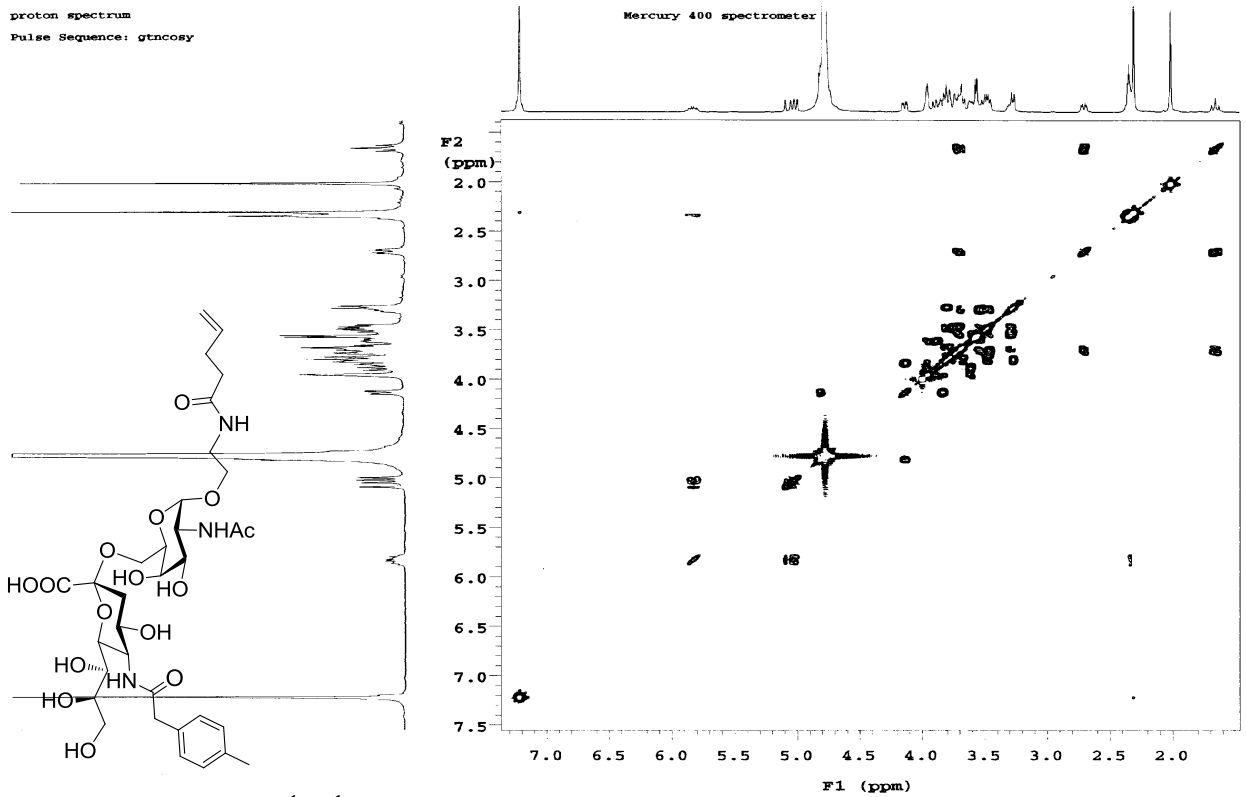


Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
874.3073	874.3074	-0.1	-0.1	15.5	17.2	7.9	C41 H51 N O16 23Na F2
	874.3075	-0.2	-0.2	26.5	20.5	11.2	C49 H48 N O14
	874.3075	-0.2	-0.2	24.5	19.2	9.9	C45 H44 N5 O9 F4
	874.3076	-0.3	-0.3	24.5	19.3	10.0	C45 H46 N5 O11 23Na F
	874.3069	0.4	0.5	10.5	12.4	3.1	C35 H53 N3 O21 23Na
	874.3069	0.4	0.5	10.5	11.1	1.8	C35 H51 N3 O19 F3
	874.3080	-0.7	-0.8	6.5	11.7	2.4	C32 H52 N3 O20 F4
	874.3064	0.9	1.0	28.5	20.3	11.1	C48 H43 N5 O8 F3
	874.3064	0.9	1.0	28.5	20.4	11.1	C48 H45 N5 O10 23Na
	874.3062	1.1	1.3	19.5	18.9	9.6	C44 H48 N O13 F4
	874.3062	1.1	1.3	19.5	18.9	9.7	C44 H50 N O15 23Na F
	874.3085	-1.2	-1.4	11.5	15.1	5.8	C38 H52 N O17 23Na F3
	874.3086	-1.3	-1.5	22.5	20.0	10.7	C46 H49 N O15 F
	874.3087	-1.4	-1.6	20.5	18.5	9.3	C42 H47 N5 O12 23Na F2
	874.3088	-1.5	-1.7	31.5	21.3	12.0	C50 H44 N5 O10
	874.3057	1.6	1.8	14.5	16.1	6.8	C38 H50 N3 O18 F2
	874.3056	1.7	1.9	3.5	15.6	6.3	C30 H53 N3 O20 23Na F4
	874.3091	-1.8	-2.1	25.5	20.8	11.6	C48 H45 N3 O7 23Na F4
	874.3093	-2.0	-2.3	13.5	16.1	6.8	C37 H52 N3 O21
	874.3051	2.2	2.5	21.5	19.4	10.1	C43 H45 N5 O9 23Na F4
	874.3051	2.2	2.5	23.5	20.8	11.5	C47 H49 N O14 23Na
	874.3050	2.3	2.6	23.5	20.7	11.4	C47 H47 N O12 F3
	874.3096	-2.3	-2.6	7.5	10.0	0.7	C35 H53 N O18 23Na F4
	874.3098	-2.5	-2.9	18.5	19.7	10.4	C43 H50 N O16 F2
	874.3098	-2.5	-2.9	16.5	17.6	8.4	C39 H48 N5 O13 23Na F3
	874.3100	-2.7	-3.1	27.5	21.4	12.1	C47 H45 N5 O11 F
	874.3046	2.7	3.1	18.5	19.1	9.8	C41 H49 N3 O17 F
	874.3045	2.8	3.2	7.5	10.8	1.5	C33 H52 N3 O19

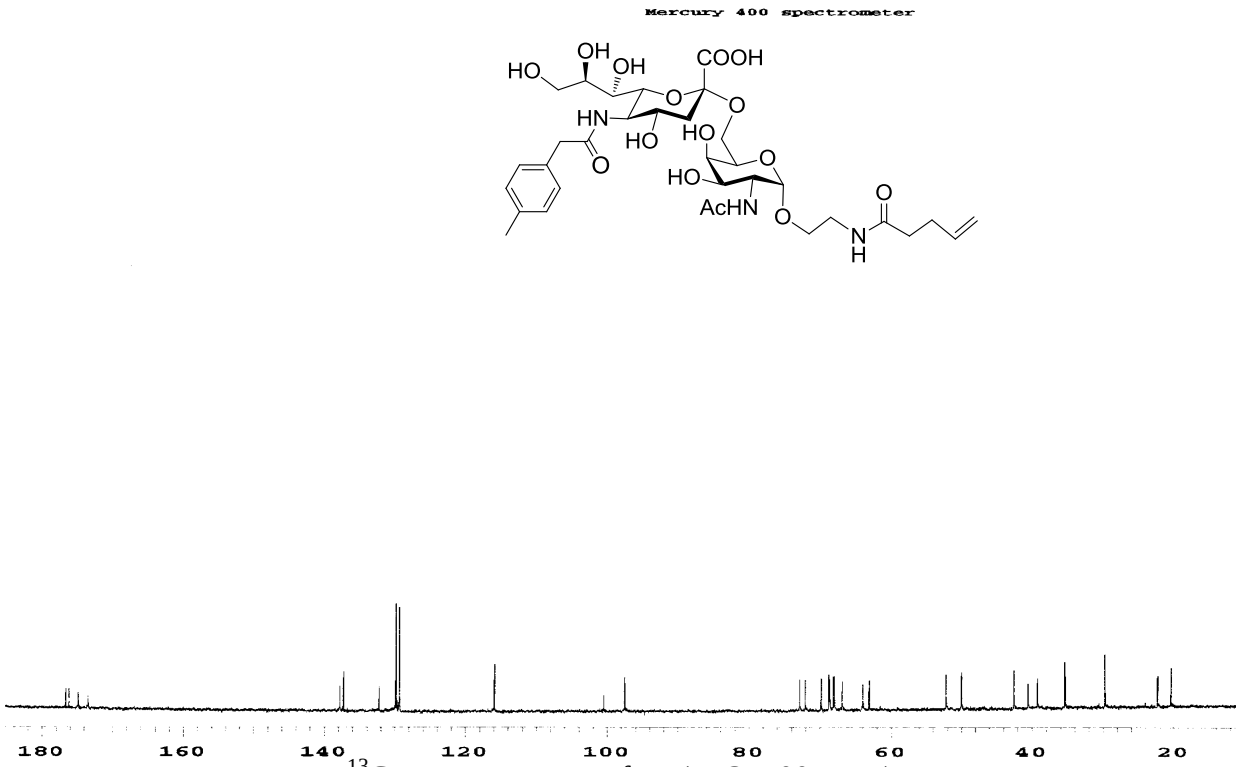
HR ESI MS spectrum of **6**



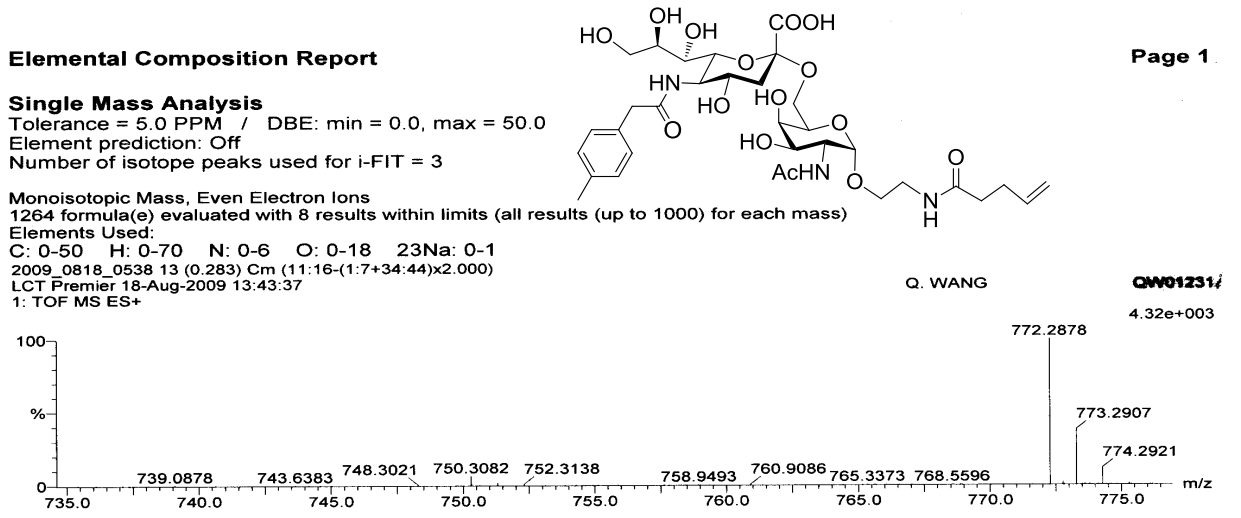
^{1}H NMR spectrum of **7a** (D_2O, 400 MHz)



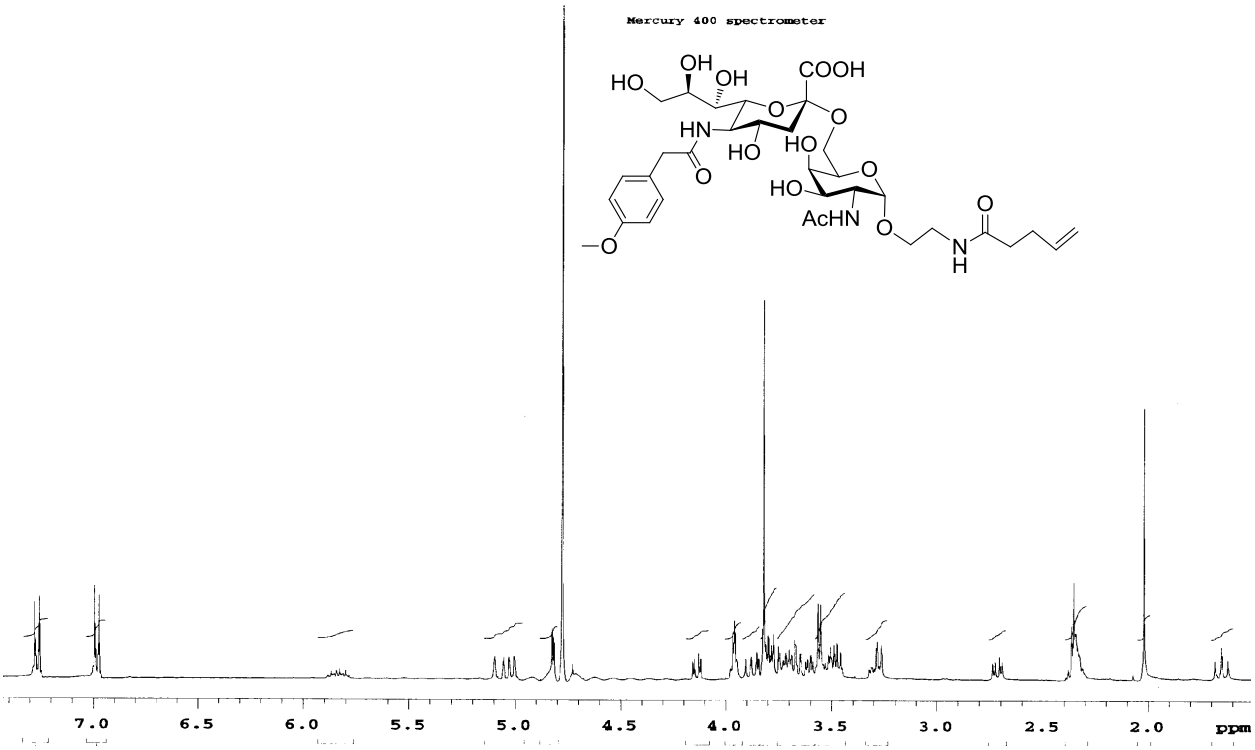
^{1}H-^{1}H COSY NMR spectrum of **7a** (D_2O, 400 MHz)



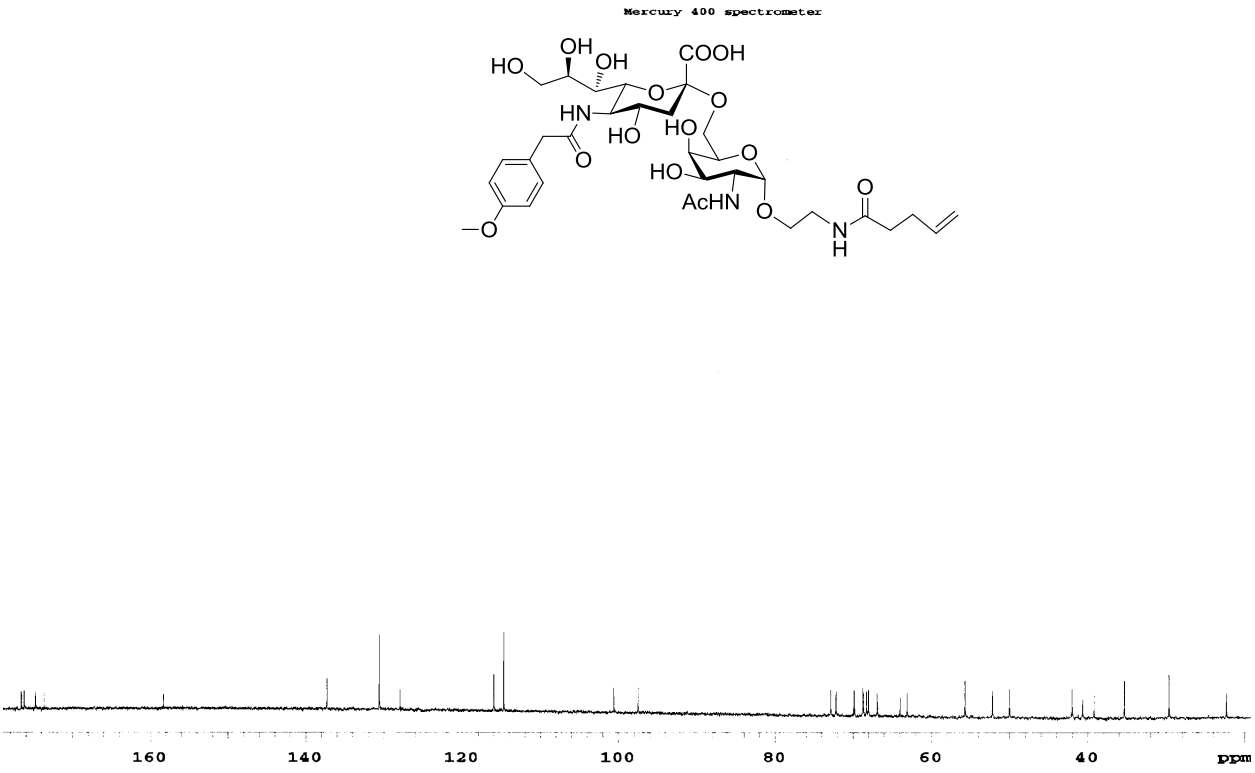
^{13}C NMR spectrum of **7a** (D_2O, 100 MHz)



HR ESI MS spectrum of **7a**

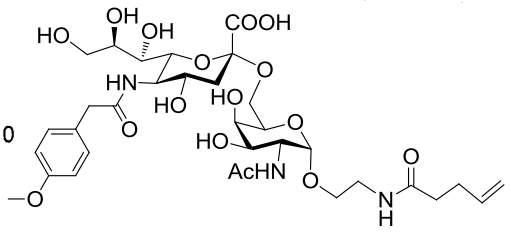


^{1}H NMR spectrum of **7b** (D_2O, 400 MHz)



^{13}C NMR spectrum of **7b** (D_2O, 100 MHz)

Elemental Composition Report **Page 1**

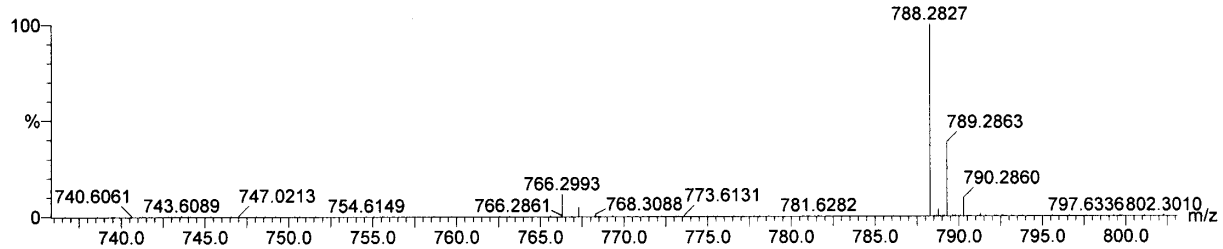


Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = 0.0, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
1857 formula(e) evaluated with 13 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-50 H: 0-70 N: 0-6 O: 0-18 23Na: 0-2
2009_0818_0539 13 (0.283) Cm (10:16-(1:8+26:35)x2.000)
LCT Premier 18-Aug-2009 14:05:41
1: TOF MS ES+

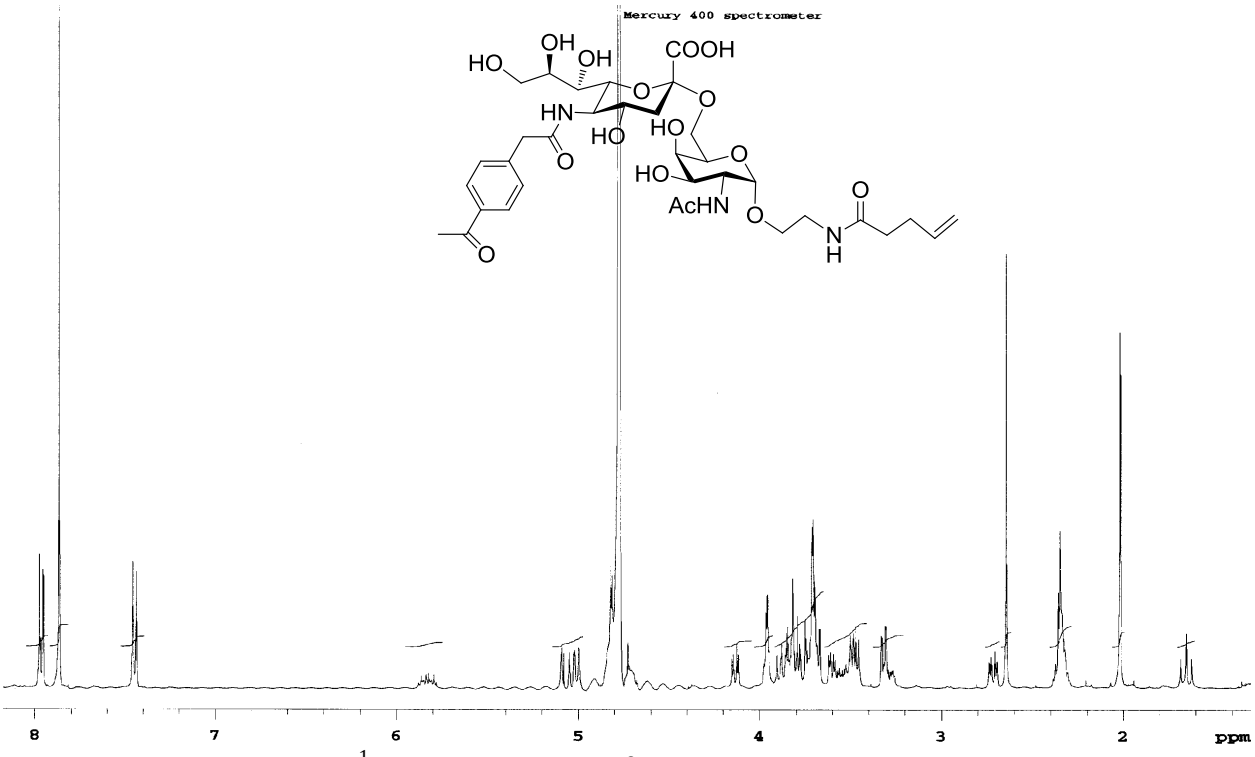
Q. WANG

5.55e+003

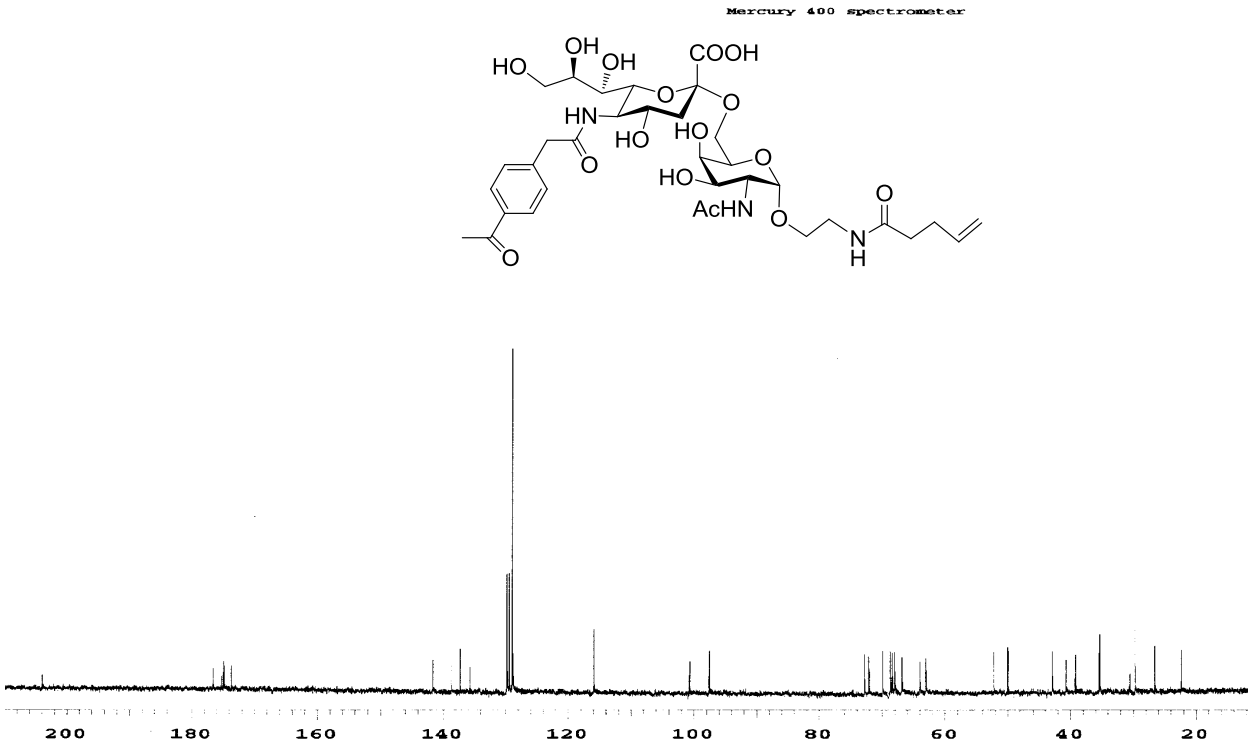


Minimum:				0.0			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
766.2993	766.2986	0.7	0.9	7.5	34.7	1.2	C31 H50 N3 O16 23Na2
	766.3011	-1.8	-2.3	10.5	34.8	1.3	C33 H49 N3 O16 23Na
	766.2994	-0.1	-0.1	9.5	34.9	1.4	C30 H48 N5 O18
	766.3027	-3.4	-4.4	11.5	36.2	2.7	C36 H50 N O14 23Na2
	766.2970	2.3	3.0	6.5	36.6	3.1	C28 H49 N5 O18 23Na
	766.2992	0.1	0.1	23.5	37.7	4.2	C45 H45 N O9 23Na
	766.2976	1.7	2.2	22.5	37.8	4.3	C42 H44 N3 O11
	766.2981	1.2	1.6	25.5	37.9	4.4	C44 H42 N5 O5 23Na2
	766.3005	-1.2	-1.6	28.5	38.3	4.8	C46 H41 N5 O5 23Na
	766.2968	2.5	3.3	20.5	38.3	4.8	C43 H46 N O9 23Na2
	766.3016	-2.3	-3.0	26.5	39.1	5.6	C47 H44 N O9
	766.3022	-2.9	-3.8	29.5	39.8	6.3	C49 H42 N3 O3 23Na2
	766.3029	-3.6	-4.7	31.5	40.5	6.9	C48 H40 N5 O5

HR ESI MS spectrum of **7b**

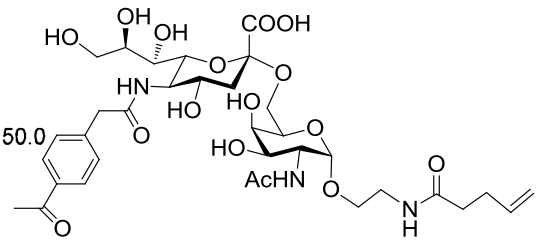


^{1}H NMR spectrum of **7c** (D_2O, 400 MHz)



^{13}C NMR spectrum of **7c** (D_2O, 100 MHz)

Elemental Composition Report

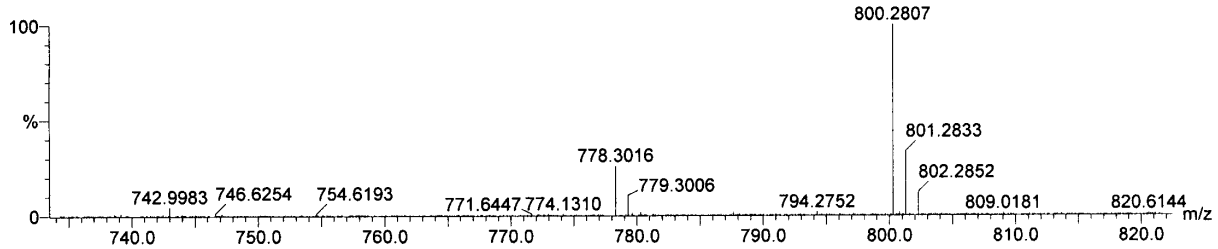


Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = 0.0, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
3403 formula(e) evaluated with 17 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-100 H: 0-100 N: 0-10 O: 0-20 23Na: 0-1
2009_0821_0548 14 (0.284) Cm (11:17-(2:8+28:37)x2.000)
LCT Premier 21-Aug-2009 13:44:33
1: TOF MS ES+

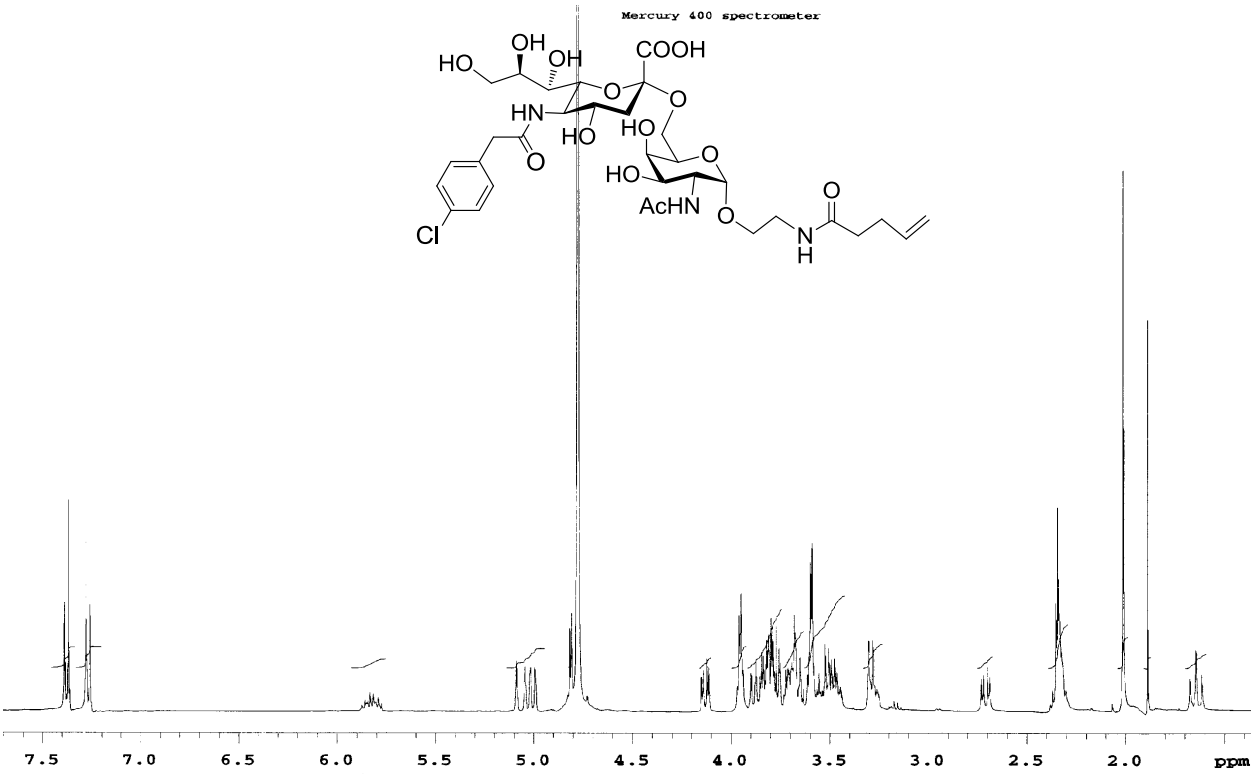
Q. WANG [redacted]

1.73e+003

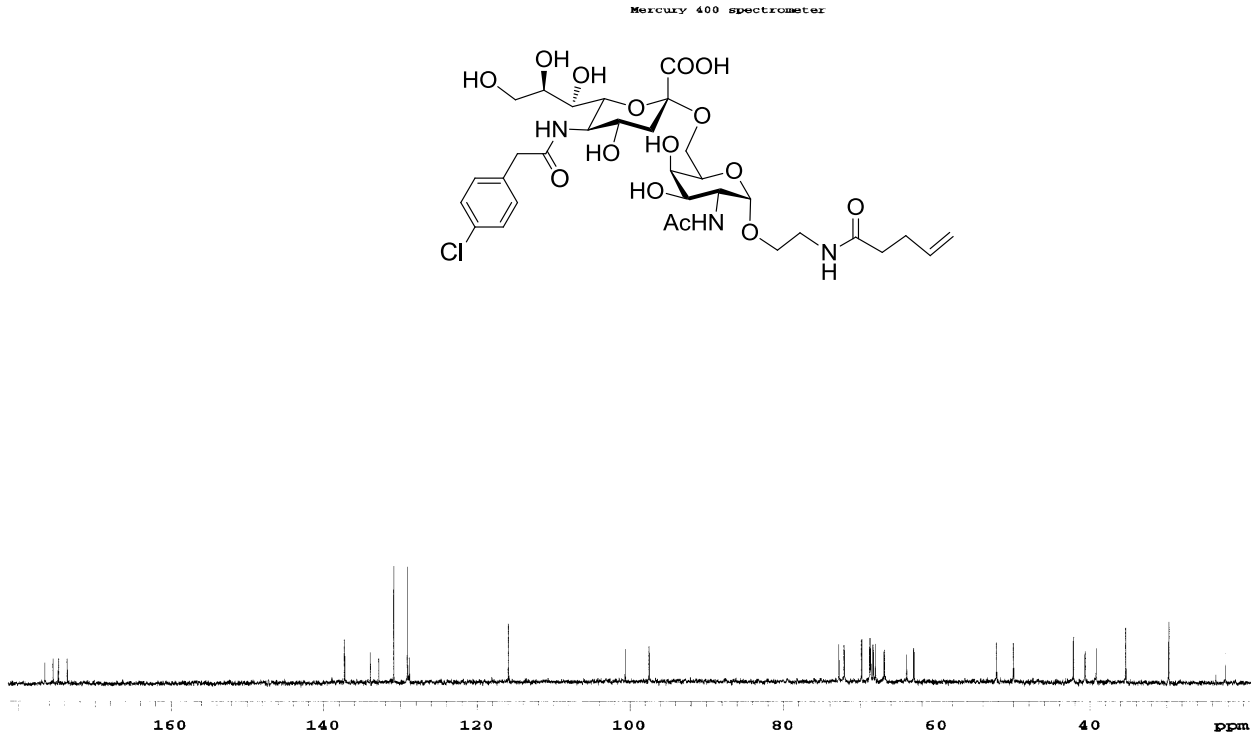


Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
Minimum:				0.0			
Maximum:		5.0	5.0	50.0			
778.3016	778.3016	0.0	0.0	27.5	91.3	5.1	C48 H44 N O9
	778.3019	-0.3	-0.4	34.5	91.2	5.0	C48 H37 N9 O 23Na
	778.3011	0.5	0.6	11.5	88.3	2.1	C34 H49 N3 O16 23Na
	778.3008	0.8	1.0	15.5	87.6	1.4	C32 H44 N9 O14
	778.3024	-0.8	-1.0	16.5	88.7	2.5	C35 H45 N7 O12 23Na
	778.3005	1.1	1.4	29.5	90.7	4.5	C47 H41 N5 O5 23Na
	778.3029	-1.3	-1.7	32.5	92.1	5.9	C49 H40 N5 O5
	778.3035	-1.9	-2.4	14.5	90.0	3.8	C36 H48 N3 O16
	778.2994	2.2	2.8	10.5	87.9	1.7	C31 H48 N5 O18
	778.2992	2.4	3.1	24.5	90.7	4.5	C46 H45 N O9 23Na
	778.3042	-2.6	-3.3	3.5	89.5	3.3	C23 H49 N9 O19 23Na
	778.2989	2.7	3.5	28.5	90.5	4.3	C44 H40 N7 O7
	778.3043	-2.7	-3.5	37.5	93.0	6.8	C50 H36 N9 O
	778.3046	-3.0	-3.9	33.5	93.5	7.3	C52 H41 N3 O3 23Na
	778.3048	-3.2	-4.1	19.5	91.0	4.7	C37 H44 N7 O12
	778.2984	3.2	4.1	12.5	87.6	1.4	C30 H45 N9 O14 23Na
	778.3051	-3.5	-4.5	15.5	91.5	5.3	C39 H49 N O14 23Na

HR ESI MS spectrum of **7c**



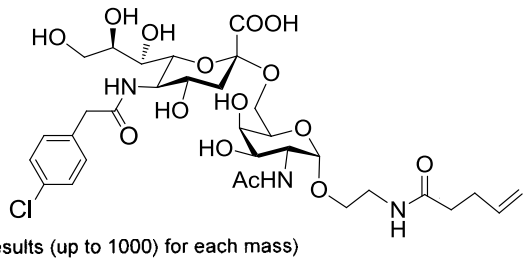
^{1}H NMR spectrum of **7d** (D_2O, 400 MHz)



^{13}C NMR spectrum of **7d** (D_2O, 100 MHz)

Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = 0.0, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

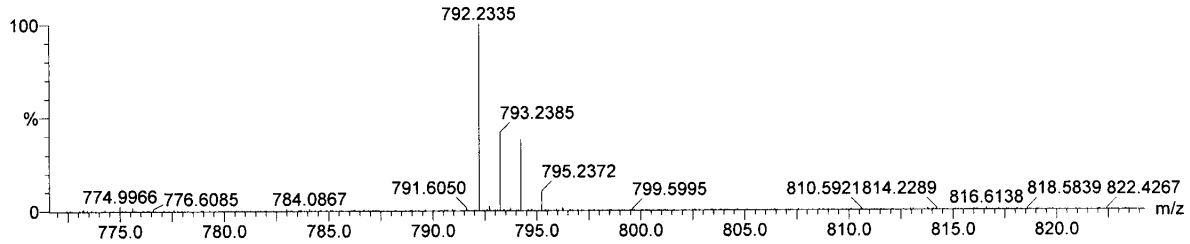


Monoisotopic Mass, Even Electron Ions
8127 formula(e) evaluated with 45 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-100 H: 0-100 N: 0-5 O: 0-20 23Na: 0-2 Cl: 0-2

2009_0825_0555a 12 (0.263) Cm (10:15-(1:6+38:45)x2.000)
LCT Premier 25-Aug-2009 16:07:38
1: TOF MS ES+

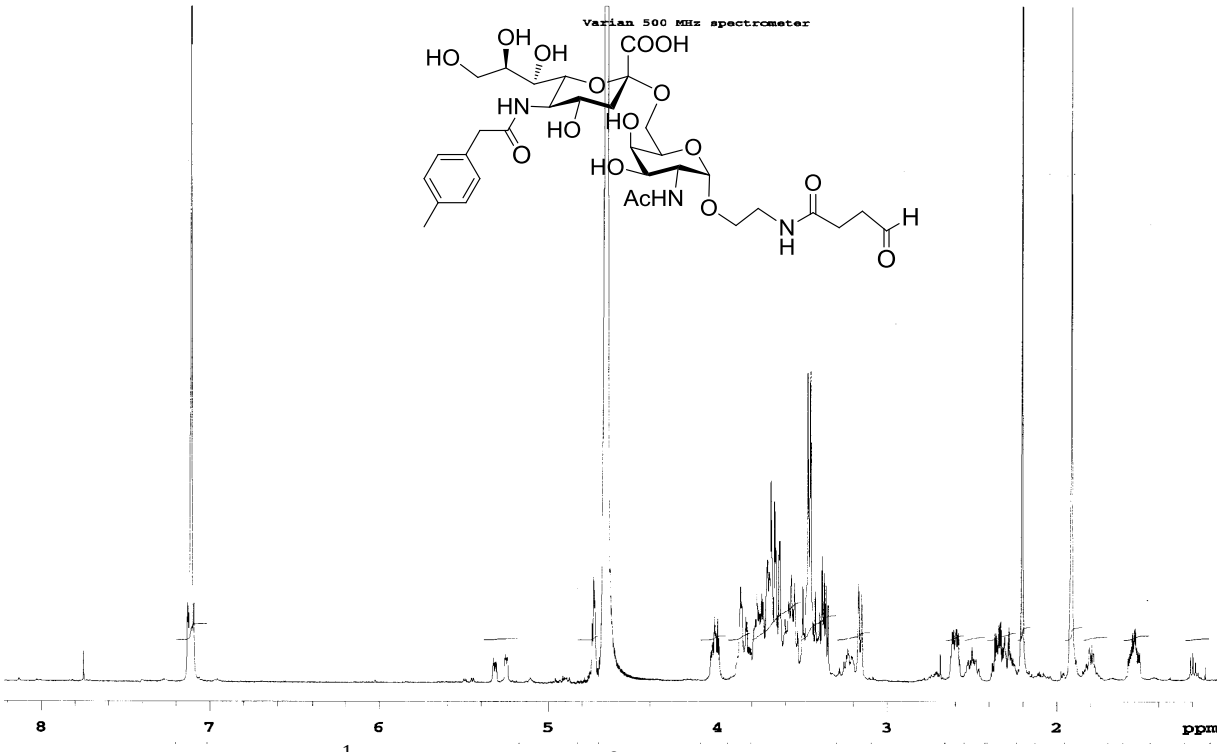
QIANLI WANG [redacted]

1.59e+003



Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
Minimum:				0.0			
Maximum:		5.0	5.0	50.0			
792.2335	792.2335	0.0	0.0	10.5	115.8	1.7	C32 H45 N3 O15 23Na2 Cl ← [redacted]
	792.2337	-0.2	-0.3	4.5	123.2	9.1	C28 H49 N3 O17 23Na Cl2
	792.2338	-0.3	-0.4	32.5	121.7	7.6	C50 H36 N O6 23Na2
	792.2332	0.3	0.4	22.5	122.8	8.6	C41 H41 N5 O6 23Na Cl2
	792.2340	-0.5	-0.6	18.5	121.4	7.2	C35 H39 N5 O15 23Na
	792.2340	-0.5	-0.6	26.5	117.2	3.0	C46 H40 N O8 23Na Cl
	792.2329	0.6	0.8	28.5	117.8	3.6	C45 H37 N5 O4 23Na2 Cl
	792.2342	-0.7	-0.9	20.5	122.4	8.3	C42 H44 N O10 Cl2
	792.2342	-0.7	-0.9	12.5	115.9	1.8	C31 H43 N5 O17 Cl
	792.2327	0.8	1.0	47.5	122.0	7.8	C61 H30 N O
	792.2327	0.8	1.0	13.5	121.4	7.2	C34 H43 N O19 23Na
	792.2346	-1.1	-1.4	34.5	121.8	7.6	C49 H34 N3 O8
	792.2324	1.1	1.4	25.5	117.5	3.3	C43 H39 N3 O10 Cl
	792.2322	1.3	1.6	31.5	121.7	7.5	C47 H35 N3 O8 23Na
	792.2348	-1.3	-1.6	23.5	122.2	8.1	C44 H42 N3 O4 23Na2 Cl2
	792.2321	1.4	1.8	3.5	124.1	9.9	C25 H48 N5 O19 Cl2
	792.2351	-1.6	-2.0	16.5	121.7	7.6	C36 H42 N O19
	792.2351	-1.6	-2.0	37.5	122.2	8.0	C51 H32 N5 O2 23Na2
	792.2318	1.7	2.1	9.5	117.8	3.6	C29 H44 N5 O17 23Na Cl
	792.2318	1.7	2.1	17.5	123.4	9.2	C40 H45 N O10 23Na Cl2
	792.2353	-1.8	-2.3	5.5	122.8	8.7	C31 H50 N O15 23Na2 Cl2
	792.2316	1.9	2.4	15.5	122.0	7.9	C33 H40 N5 O15 23Na2
	792.2354	-1.9	-2.4	31.5	117.6	3.5	C47 H36 N5 O4

HR ESI MS spectrum of **7d**



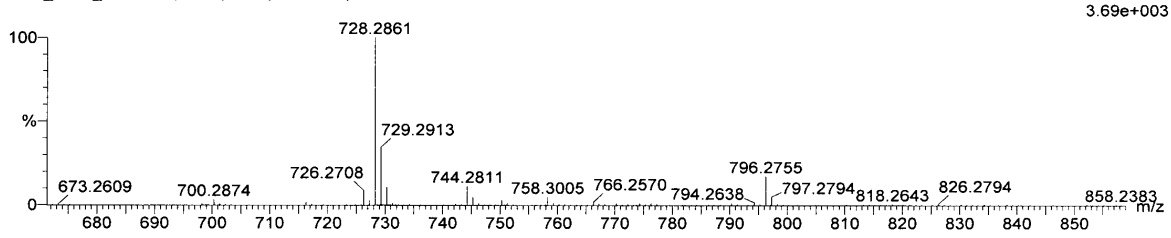
^{1}H NMR spectrum of **8a** (D_2O, 500 MHz)

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = 0.0, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

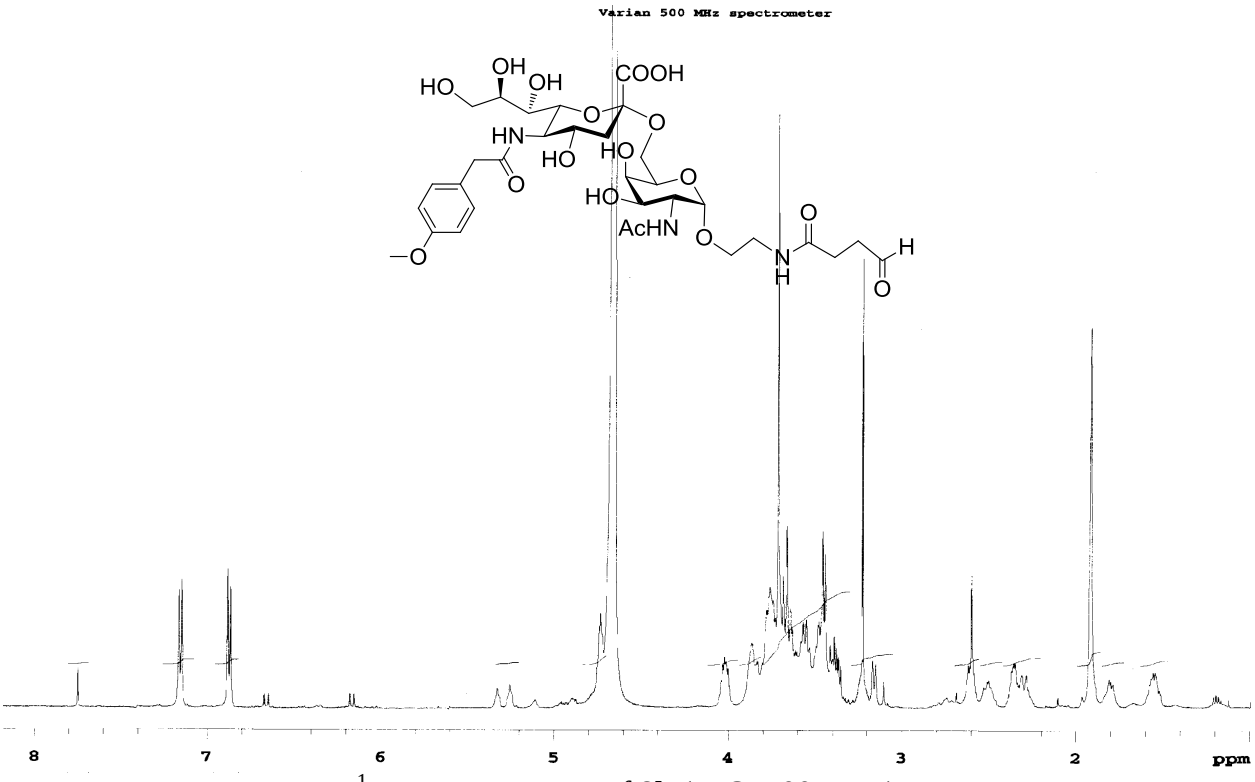
Monoisotopic Mass, Even Electron Ions
1511 formula(e) evaluated with 6 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-100 H: 0-200 N: 0-4 O: 0-20 Na: 0-1
QIANLI WANG QW01237
Lew 2008-07b.pro
2009_0921_0594 14 (0.283) Cm (12:17-3:8)

LCT Premier 21-Sep-2009 10:36:00
1: TOF MS ES-
3.69e+003



Minimum:				0.0			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
728.2861	728.2860	0.1	0.1	24.5	90.7	6.4	C44 H42 N O9
	728.2854	0.7	1.0	8.5	84.7	0.4	C30 H47 N3 O16 Na
	728.2878	-1.7	-2.3	11.5	85.6	1.2	C32 H46 N3 O16
	728.2836	2.5	3.4	21.5	91.3	7.0	C42 H43 N O9 Na
	728.2889	-2.8	-3.8	30.5	92.7	8.4	C48 H39 N3 O3 Na
	728.2894	-3.3	-4.5	12.5	88.4	4.1	C35 H47 N O14 Na

HR ESI MS spectrum of **8a**



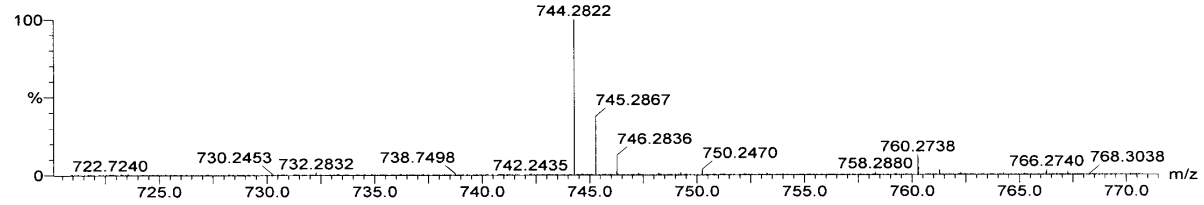
^{1}H NMR spectrum of **8b** (D_2O, 500 MHz)

Elemental Composition Report Page 1

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = 0.0, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

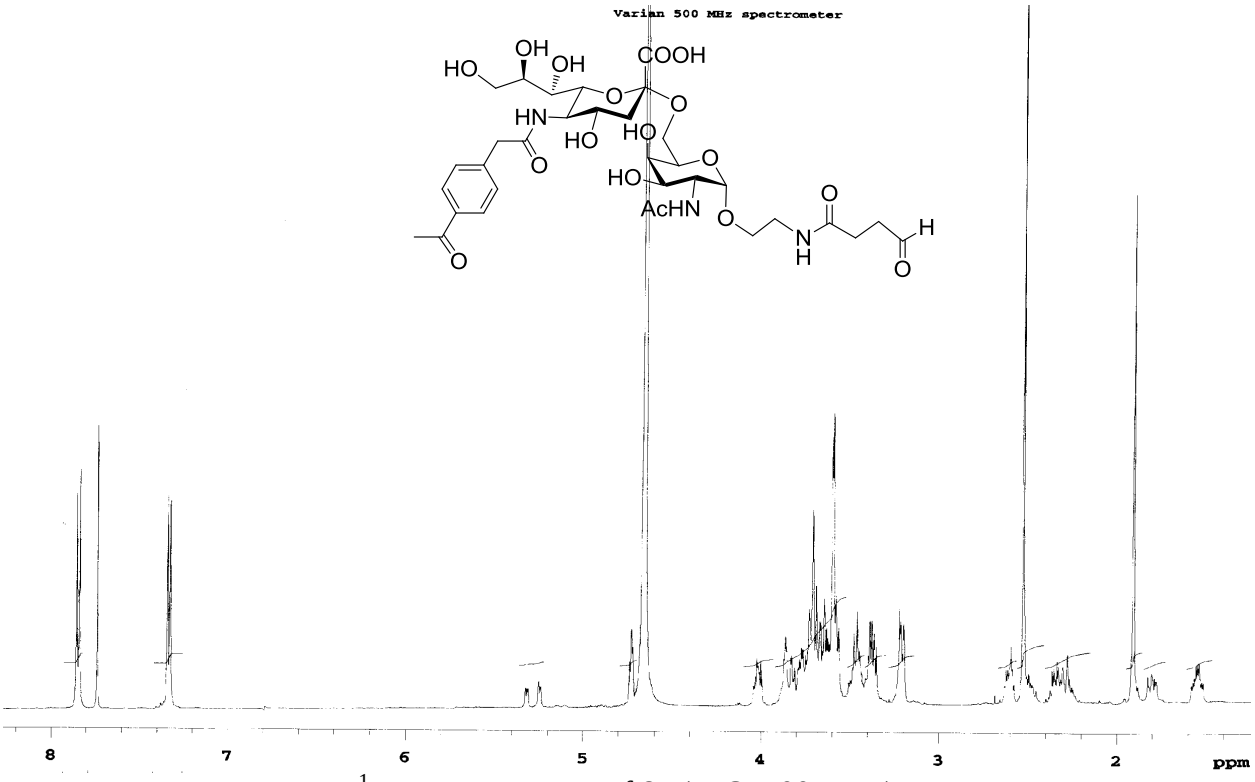
Monoisotopic Mass, Even Electron Ions
1551 formula(e) evaluated with 5 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-100 H: 0-200 N: 0-4 O: 0-20 Na: 0-1
QIANLI WANG QW01238
Lew 2008-07b.pro
2009_0921_0595 15 (0.300) Cm (13:20-3:9)

LCT Premier 21-Sep-2009 10:55:38
1: TOF MS ES-
8.31e+002



Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
Minimum:				0.0			
Maximum:		5.0	5.0	100.0			
744.2822	744.2827	-0.5	-0.7	11.5	121.8	0.5	C32 H46 N3 O17
	744.2809	1.3	1.7	24.5	125.5	4.2	C44 H42 N O10
	744.2838	-1.6	-2.1	30.5	127.0	5.7	C48 H39 N3 O4 Na
	744.2803	1.9	2.6	8.5	122.9	1.6	C30 H47 N3 O17 Na
	744.2843	-2.1	-2.8	12.5	122.9	1.6	C35 H47 N O15 Na

HR ESI MS spectrum of **8b**



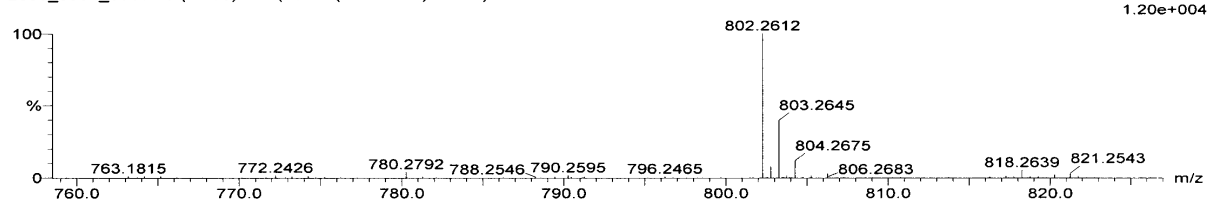
^{1}H NMR spectrum of **8c** (D_2O, 500 MHz)

Elemental Composition Report Page 1

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = 0.0, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

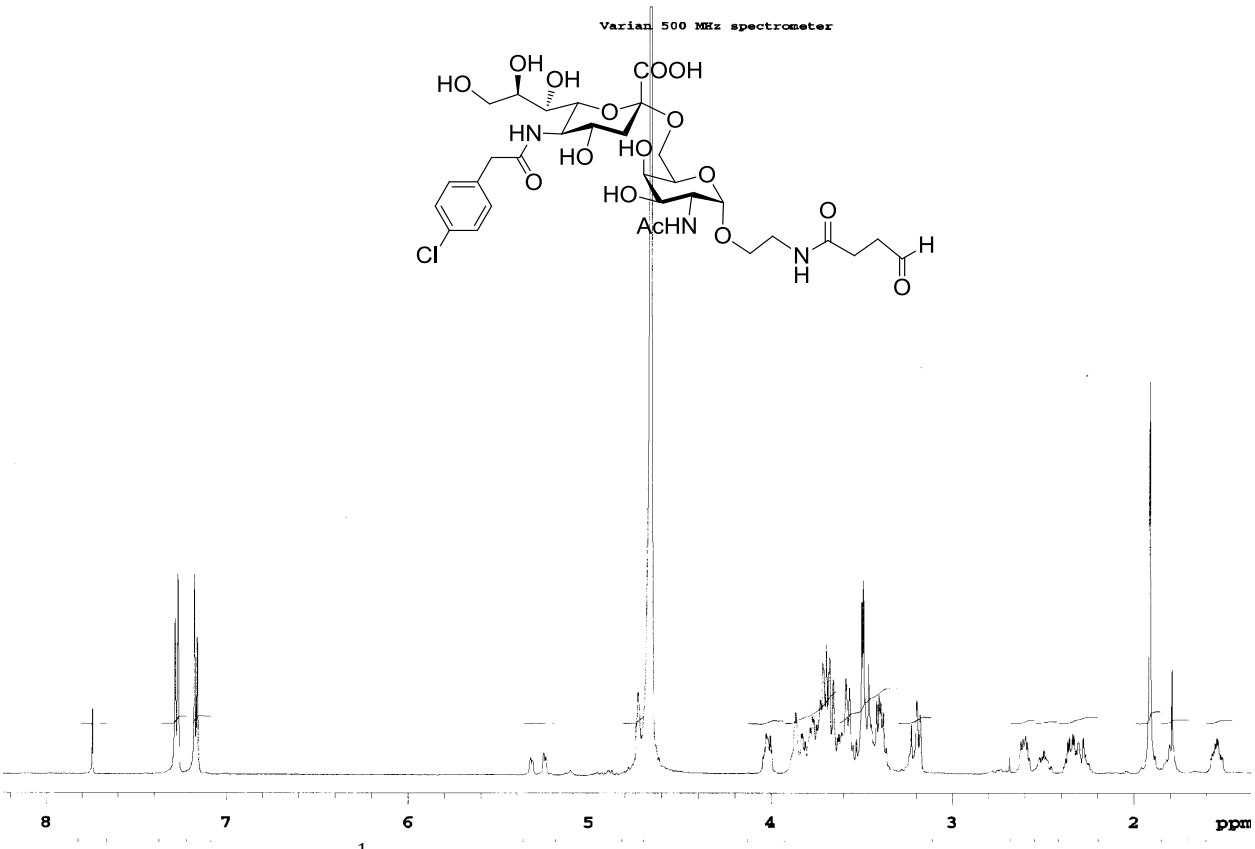
Monoisotopic Mass, Even Electron Ions
2755 formula(e) evaluated with 20 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-50 H: 0-60 N: 0-10 O: 0-20 23Na: 0-2
Q. WANG QW01239
Lew 2008-07b.pro
2009_0901_0563 13 (0.283) Cm (10:19-(1:6+32:45)x2.000)

LCT Premier 01-Sep-2009 11:08:58
1: TOF MS ES+
1.20e+004



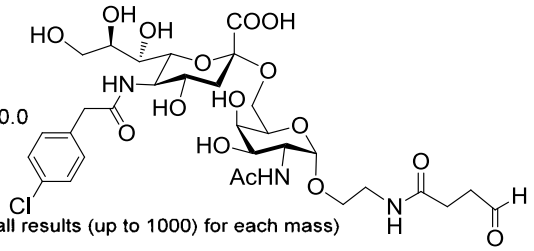
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
Minimum:				0.0			
Maximum:		5.0	5.0	50.0			
802.2612	802.2612	0.0	0.0	26.5	154.4	8.9	C44 H40 N3 O12
	802.2617	-0.5	-0.6	29.5	155.0	9.5	C46 H38 N5 O6 23Na2
	802.2606	0.6	0.7	10.5	149.8	4.3	C30 H45 N5 O19 23Na
	802.2604	0.8	1.0	24.5	154.6	9.0	C45 H42 N O10 23Na2
	802.2620	-0.8	-1.0	15.5	148.1	2.6	C31 H41 N9 O15 23Na
	802.2623	-1.1	-1.4	11.5	146.4	0.9	C33 H46 N3 O17 23Na2
	802.2601	1.1	1.4	28.5	154.7	9.2	C43 H37 N7 O8 23Na

HR ESI MS spectrum of **8c**



^{1}H NMR spectrum of **8d** (D_2O, 500 MHz)

Elemental Composition Report **Page 1**

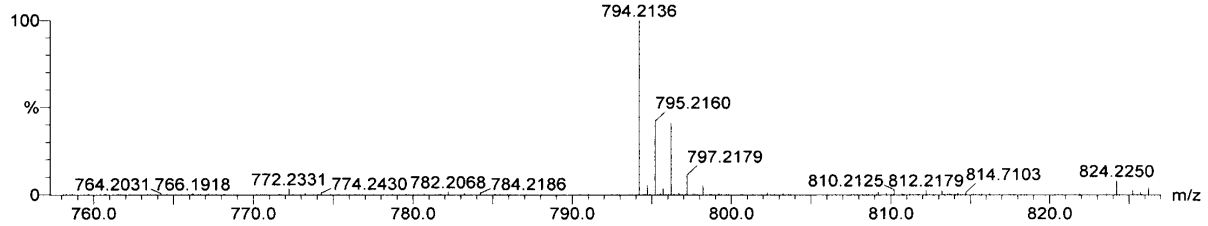


Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = 0.0, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
9211 formula(e) evaluated with 70 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-50 H: 0-60 N: 0-10 O: 0-20 23Na: 0-2 Cl: 0-2

QIANLI WANG **QW01240**
Lew 2008-07b.pro
2009_0901_0564 13 (0.283) Cm (11:18-(2:8+30:38)x2.000)

LCT Premier 01-Sep-2009 11:21:08
1: TOF MS ES+
4.58e+003



Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
Minimum:				0.0			
Maximum:		5.0	5.0	50.0			
794.2136	794.2135	0.1	0.1	20.5	148.5	10.9	C41 H42 N O11 Cl2
	794.2135	0.1	0.1	33.5	143.0	5.4	C45 H31 N9 O 23Na2 Cl
	794.2135	0.1	0.1	12.5	141.5	3.9	C30 H41 N5 O18 Cl
	794.2138	-0.2	-0.3	27.5	148.1	10.5	C41 H35 N9 O3 23Na Cl2
	794.2139	-0.3	-0.4	34.5	152.2	14.6	C48 H32 N3 O9
	794.2133	0.3	0.4	26.5	142.5	4.9	C45 H38 N O9 23Na Cl
	794.2133	0.3	0.4	18.5	151.9	14.3	C34 H37 N5 O16 23Na
	794.2140	-0.4	-0.5	23.5	148.3	10.7	C43 H40 N3 O5 23Na2 Cl2
	794.2141	-0.5	-0.6	15.5	138.9	1.3	C32 H39 N7 O12 23Na2 Cl
	794.2131	0.5	0.6	32.5	152.2	14.6	C49 H34 N O7 23Na2
	794.2130	0.6	0.8	30.5	142.4	4.8	C43 H33 N7 O7 Cl
	794.2143	-0.7	-0.9	9.5	149.0	11.4	C28 H43 N7 O14 23Na Cl2
	794.2129	0.7	0.9	4.5	149.6	12.0	C27 H47 N3 O18 23Na Cl2
	794.2144	-0.8	-1.0	37.5	152.4	14.8	C50 H30 N5 O3 23Na2
	794.2144	-0.8	-1.0	16.5	152.2	14.5	C35 H40 N O20
	794.2128	0.8	1.0	36.5	151.9	14.3	C47 H29 N7 O5 23Na
	794.2127	0.9	1.1	10.5	141.5	3.9	C31 H43 N3 O16 23Na2 Cl
	794.2127	0.9	1.1	8.5	149.6	12.0	C25 H42 N9 O16 Cl2
	794.2146	-1.0	-1.3	31.5	143.3	5.7	C46 H34 N5 O5 23Na Cl
	794.2146	-1.0	-1.3	23.5	152.1	14.5	C35 H33 N9 O12 23Na
	794.2146	-1.0	-1.3	5.5	149.3	11.7	C30 H48 N O16 23Na2 Cl2
	794.2124	1.2	1.5	22.5	148.8	11.2	C40 H39 N5 O7 23Na Cl2
	794.2148	-1.2	-1.5	25.5	148.4	10.8	C42 H38 N5 O7

HR ESI MS spectrum of **8d**